COMPARISON OF EFFICACY OF AUTOLOGOUS PLATELET RICH FIBRIN (PRF) OVER MOIST STERILE SALINE DRESSING IN CHRONIC VENOUS LEG ULCERS - A RANDOMIZED CONTROL TRIAL.

Dissertation Submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

In the fulfilment of the regulations for the award of the degree

M.D.

DERMATOLOGY, VENEREOLOGY AND LEPROLOGY

DEPARTMENT OF DERMATOLOGY, VENEROLOGY AND LEPROLOGY

PSG INSTITUTE OF MEDICAL SCIENCE AND RESEARCH

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

CHENNAI, TAMILNADU

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GUIDE

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CERTIFICATE

This is to certify that the thesis entitled **"COMPARISON OF EFFICACY OF AUTOLOGOUS PLATELET RICH FIBRIN (PRF) OVER MOIST STERILE SALINE DRESSING IN CHRONIC VENOUS LEG ULCERS - A RANDOMIZED CONTROL TRIAL"** is a bonafide work of **Dr. ANIRUDH SOMANI** done under the direct guidance and supervision of **Dr. REENA RAI, MD,** in the department of Dermatology, Venereology and Leprology, PSG Institute of Medical Sciences and Research, Coimbatore in fulfillment of the regulations of Dr.MGR Medical University for the award of M.D. degree in Dermatology, Venereology and Leprology.

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DEAN

DECLARATION

I hereby declare that this dissertation entitled **"COMPARISON OF EFFICACY OF AUTOLOGOUS PLATELET RICH FIBRIN (PRF) OVER MOIST STERILE SALINE DRESSING IN CHRONIC VENOUS LEG ULCERS - A RANDOMIZED CONTROL TRIAL"** was prepared by me under the direct guidance and supervision of **Dr. REENA RAI, MD,** PSG Institute of Medical Sciences and Research, Coimbatore.

The dissertation is submitted to the Tamilnadu Dr.MGR Medical University in fulfillment of the University regulation for the award of M.D. degree in Dermatology, Venereology and Leprology. This dissertation has not been submitted for the award of any other Degree or Diploma.

Dr. ANIRUDH SOMANI

CERTIFICATE BY THE GUIDE

This is to certify that the thesis entitled **““COMPARISON OF EFFICACY OF AUTOLOGOUS PLATELET RICH FIBRIN (PRF) OVER MOIST STERILE SALINE DRESSING IN CHRONIC VENOUS LEG ULCERS - A RANDOMIZED CONTROL TRIAL”** is a bonafide work of **Dr. ANIRUDH SOMANI** done under my direct guidance and supervision in the department of Dermatology, Venereology and Leprology, PSG Institute of Medical Sciences and Research, Coimbatore in the fulfillment of the regulations of Dr.MGR Medical University for the award of MD degree in Dermatology, Venereology and Leprology.

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Institutional Human Ethics Committee

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To
Dr Anirudh Somani
Postgraduate
Department of Dermatology
PSG IMS & R
Coimbatore

Ref: Project No. 14/416

Date: April 9, 2015

Dear Dr Anirudh Somani,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 09.01.2015 to conduct the research study entitled "Efficacy of autologous platelet rich fibrin (PRF) over moist sterile saline dressing in chronic venous leg ulcers" during the IHEC review held on 24.02.2015.

The following documents were reviewed and approved:

1. Project Submission form
2. Study protocol
3. Informed consent form
4. Data collection tool
5. Current CVs of Principal investigator, Co-investigators
6. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 24.02.2015 at College Council Room, PSG IMS & R between 2.00 pm and 4.30 pm:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mrs Y Ashraf	MPT	Physiotherapy	Female	Yes	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Mr Gowpathy Velappan	BA., BL	Legal Advisor	Male	No	Yes
4	Mr P Karuppuchamy	M Phil in PSW	Social Scientist	Male	Yes	Yes
5	Mrs G Malarvizhi	M Sc	Nursing	Female	Yes	Yes
6	Mr. R. Nandakumar (Vice-Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
7	Dr. G. Rajendiran	DM	Clinician (Cardiology)	Male	Yes	No

8	Dr. V. Ramamurthy	Ph D	Biotechnology	Male	Yes	No
9	Mrs P Rama	M Pharm	Non-Medical (Pharmacy)	Female	Yes	Yes
10	Dr. P. Sathyan (Chairperson, IHEC)	DO, DNB	Clinician (Ophthalmology)	Male	No	No
11	Dr. Seetha Panicker	MD	Clinician (Obstetrics & Gyanaecology)	Female	Yes	Yes
12	Dr. S. Shanthakumari	MD	Pathology, Ethicist	Female	Yes	No
13	Dr. Sudha Ramalingam (Alternate Member-Secretary, IHEC)	MD	Public Health, Epidemiology, Genetics, Ethicist	Female	Yes	No
14	Mrs. Swasthika Soundararaj	MBA	Lay person	Female	No	Yes
15	Dr. D. Vijaya	M Sc, Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

Following points must be noted:

1. IHEC should be informed of the date of initiation of the study
2. Status report of the study should be submitted to the IHEC every 12 months
3. PI and other investigators should co-operate fully with IHEC, who will monitor the trial from time to time
4. At the time of PI's retirement/intention to leave the institute, study responsibility should be transferred to a colleague after obtaining clearance from HOD, Status report, including accounts details should be submitted to IHEC and extramural sponsors
5. In case of any new information or any SAE, which could affect any study, must be informed to IHEC and sponsors. The PI should report SAEs occurred for IHEC approved studies within 7 days of the occurrence of the SAE. If the SAE is 'Death', the IHEC Secretariat will receive the SAE reporting form within 24 hours of the occurrence
6. In the event of any protocol amendments, IHEC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. Alteration in the budgetary status should be clearly indicated and the revised budget form should be submitted
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval
   d. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented
   e. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IHEC

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and only then can they be implemented

f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review

7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

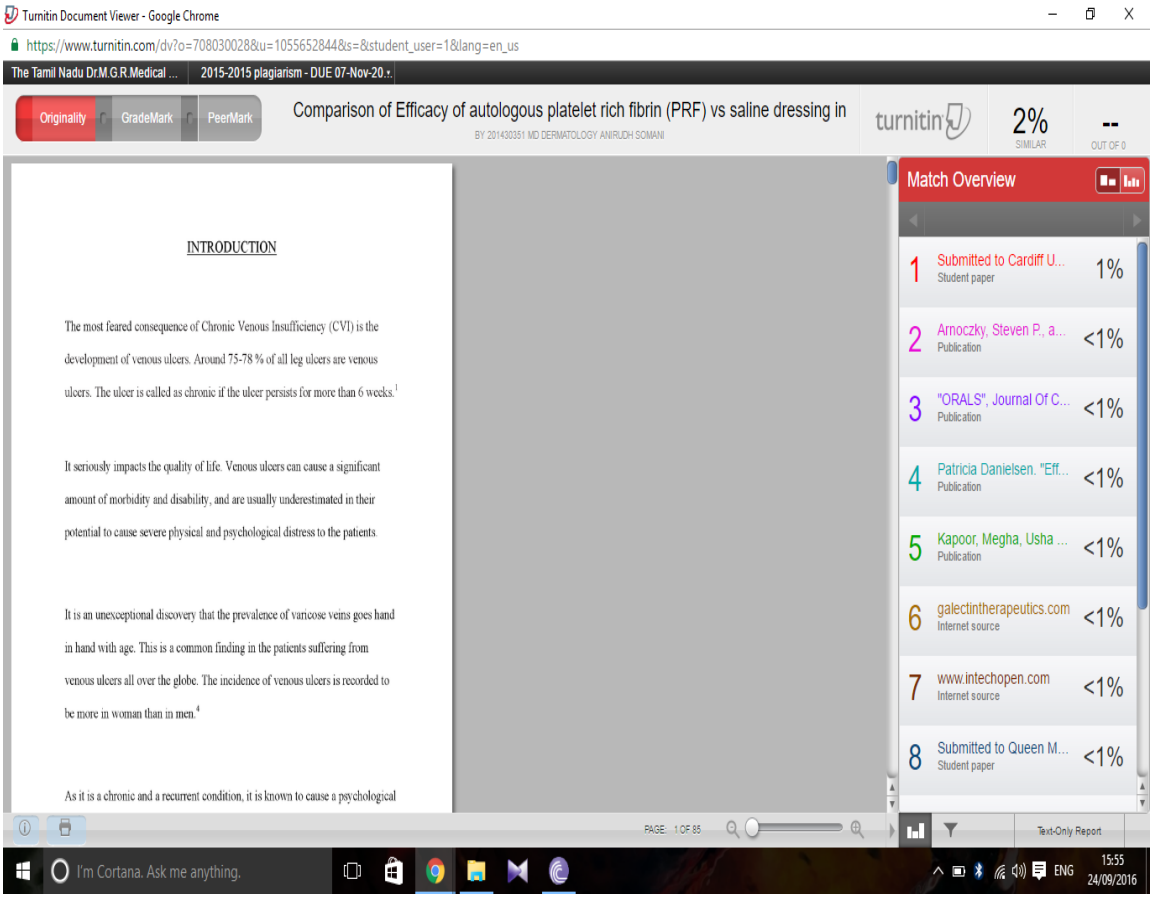
Thanking You,

Yours Sincerely,

SECRETARY
PSG IMS&R
COIMBATORE-641004.
INSTITUTIONAL HUMAN ETHICS COMMITTEE

Dr S Bhuvaneshwari
Member-Secretary
Institutional Human Ethics Committee

PLAGIARISM TOOL



ACKNOWLEDGEMENT

The successful completion of my dissertation would not have been possible without the contribution of many people to whom, I would like to express my deep sense of gratitude.

First and foremost, I am very grateful to my guide, Dr.Reena Rai, for her timely advice and guidance at various stages of my dissertation.

I take great pleasure in thanking my Professor, Dr.C.R.Srinivas, who has been an extraordinary mentor, for the kind of inspiration and scholarly guidance which have helped me build a strong foundation in the subject.

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I would take this as a great opportunity to thank all my patients without whose consent, I would not have been able to complete this study.

Above all, I would like to exceptionally thank Dr.Ashwini Annabathula for always being there for me and for being a constant source of support and motivation throughout the period of my post-graduation and Dr.Subha Ranjani for being a true friend and for always lending a helping hand, especially when language was a barrier.

I would be failing in my duty if I do not immensely thank my beloved parents for making me what I am today, and my wife, Sneha, for her monumental patience, understanding and her unstinted support all along without which this thesis would not have been completed.

TABLE OF CONTENTS

INTRODUCTION

The most feared consequence of Chronic Venous Insufficiency (CVI) is the development of venous ulcers. Around 75-78 % of all leg ulcers are venous ulcers. The ulcer is called as chronic if the ulcer persists for more than 6 weeks.[1]

It seriously impacts the quality of life. Venous ulcers can cause a significant amount of morbidity and disability, and are usually underestimated in their potential to cause severe physical and psychological distress to the patients.

It is an unexceptional discovery that the prevalence of varicose veins goes hand in hand with age. This is a common finding in the patients suffering from venous ulcers all over the globe. The incidence of venous ulcers is recorded to be more in woman than in men.[4]

As it is a chronic and a recurrent condition, it is known to cause a psychological and a socio-economic impact on the patient's life. There are many studies that show that a patient of venous ulcer has far less working days than healthy individuals.[7, 8] The loss of working days and the financial burden of continued treatment has an adverse impact, not only on the patient, but also on his or her family.

The treatment for venous ulcer takes a long time and its recurrence rate is also quite high.[1,5,6.] There are various modalities of treatment available most of which deal with treating the underlying cause. But along with the treatment the patient should also be counselled about the disease and the measures that the patient can take to reduce the recurrence of the condition.

The main aim of the doctor (physician) for the treatment of venous ulcer must be to reduce the size of the ulcer and to prevent the recurrence of the ulcer.

As this is a chronic condition the ulcer is devoid of all the growth factors and nutrients required for healing. This could be one of the reasons for the ulcer to take a long time to heal.

Dressings play a major role in healing of these ulcers. Moist occlusive dressings are known to improve wound healing. Platelet concentrates also help in healing faster by supplying vital growth factors and nutrients required by the ulcer and also induce and promote wound healing. There are multiple platelet rich concentrates that are available and each type of concentrate has its own applications in medicine.

Products obtained from blood which were used to seal and heal wounds and ulcers started around 5 decades back[142]. For ulcers the platelet concentrate that was used was the Platelet Rich Fibrin.

The effectiveness of products obtained from blood in wound healing was first described by Whiteman et al.[141] following which the use of these products became extremely popular in the last 15 years.

Need For Study

This study was conducted to determine the efficacy of platelet rich fibrin to heal / reduce the size of the ulcer in comparison to the moist saline dressing so as to reduce the disability and ambulate the patient at the earliest.

AIM OF THE STUDY

PRIMARY AIM

- To compare the efficacy of autologous PRF with saline dressing in patients with chronic venous leg ulcers

SECONDARY AIM

- To compare the mean reduction in ulcer area at end of 4 weeks.

REVIEW OF LITERATURE

Venous Ulcers

The most dreaded complication of Chronic Venous Insufficiency (CVI) is venous ulcers. It accounts for about three forth of all lower leg ulcers. It is called as chronic if the ulcer persists for more than 6 weeks.[1]

Various causes implicated in the etiology of venous ulcers include:

A. Arterial insufficiency
B. Trauma
C. Rheumatoid arthritis
D. Sickle cell anaemia
E. Diabetes mellitus
F. Vasculitis
G. Osteomyelitis
H. Skin tumour

In a few cases the cause of ulceration cannot be found and in others there are multiple causes.[2]

The diseases of the venous system are not given the importance it deserves for the amount of morbidity and discomfort it causes to the

patients. It is usually seen in patients after the fourth decade of life. Its incidence is more in women when compared to men.[4]

Due to the presence of varicose veins which is a very common clinical finding in patients with venous ulcer theses ulcers are also called as “Varicose ulcers”.

Due to the gravitational pull and incompetent valves there is pooling of blood and hence it is also called as “gravitational ulcer” and “stasis ulcer”.

CVI is an underestimated cause of morbidity as it disables the patient and it is present all around the globe. Given below is the clinical classification according to the ascending severity of this condition.

CLINICAL CLASSIFICATION.[3]

Grade 0	No sign of disease
Grade 1	Telangiectasia
Grade 2	Varicosities
Grade 3	Oedema
Grade 4	Changes due to venous insufficiency (e.g. hyperpigmentation, eczema (of venous cause) and lipodermatosclerosis)
Grade 5	Changes mentioned above with recovered ulceration
Grade 6	Changes mentioned above with ulceration

It is an unexceptional discovery that in males and in females the incidence of varicose veins increases with age.

As the incidence of CVI and specially the venous ulcers is very high and causes disability and morbidity, it has a great impact on the Psychological and socioeconomic state of the patient. The patient cannot go out to earn his living and this reduces the patient's quality of life. The treatment for venous ulcer takes a long time and its recurrence rate is also quite high.[1,5,6]

To assess the disability in this condition a few studies have been conducted which state the loss of working days/hours by patients suffering from venous disease. In a study by Weiss et al. it has been shown that around 6 million work days are lost in United States of America alone.[7] A Similar outcome was stated in a study conducted in France by Lafuma et al.[8]

Other causes for the formation of venous ulcers as a consequence to CVI are

a. Age (adult onset)
b. Sex (more in females)
c. Occupation (seen more in patients who stand for a long time)
d. Obesity
e. Family history
f. Race and
g. No. of Pregnancies

The impact of these risk factors is still a topic of debate.

The incompetence of valves in the superficial, perforating or deep veins play a crucial role in the disease, but a combination of these rather than singly is always a matter of serious concern.[9]

Etiopathogenesis

The pathogenesis of venous ulcer and CVI are very closely related. The improper functioning of the muscular pump of the calf which is also known as the peripheral causes venous hypertension.[10, 11]

This peripheral hearts main function is to pump the deoxygenated blood from the lower limbs to the heart. It consists of the calf muscle, the deep, superficial and the perforating veins.

While walking the calf muscles exerts pressure (the systole) and the blood in the deep veins is pushed or pumped in the upward direction. While at rest, these muscles relax (the diastole) causing the passage of blood from the superficial into the deep veins through the perforating veins.

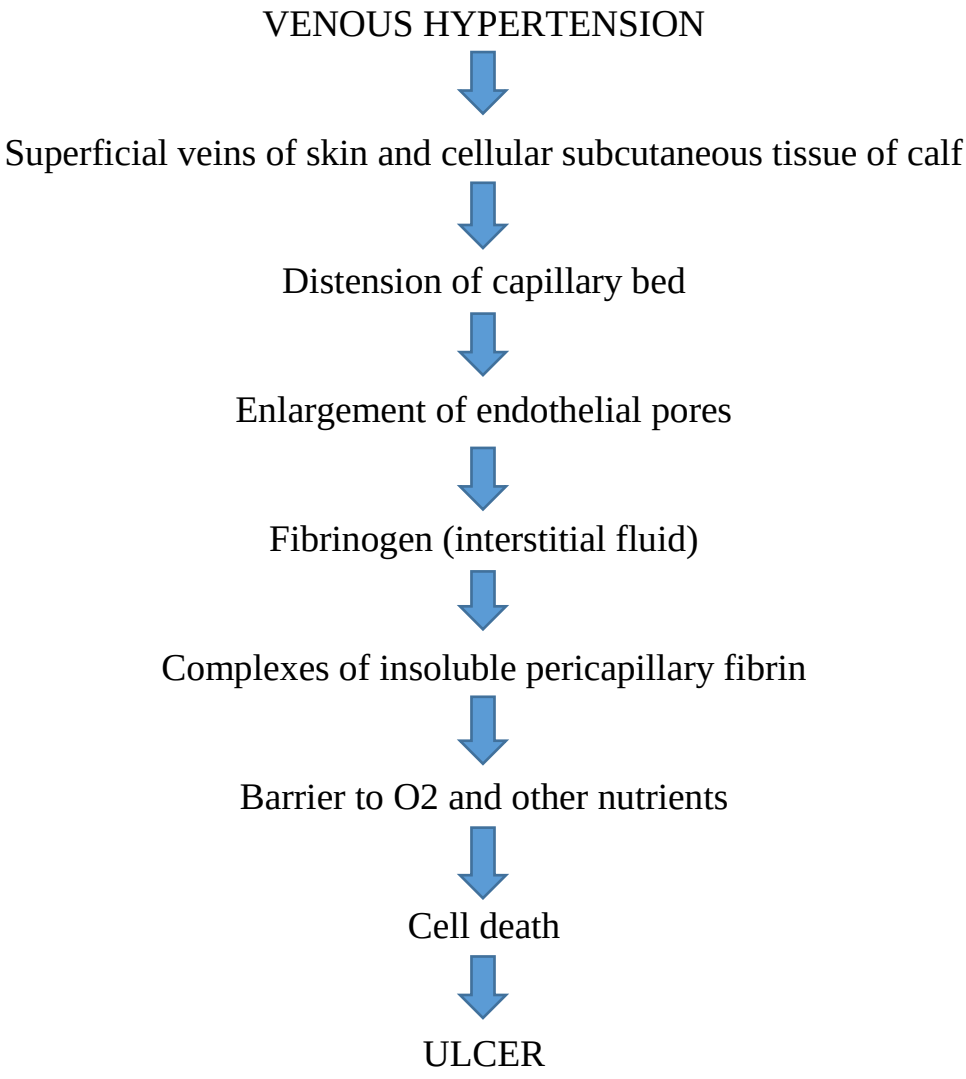
There are valves in these veins which while functioning properly prevent the backword flow of blood and maintain the flow in one direction i.e., from the superficial veins to the deeper veins through the perforating veins.[12,13]

The improper functioning of this system can be a consequence of failure of the valve of the deep veins, impediment of the deep venous system, failure of the valve of the superficial veins or the perforating veins, aterio-venous malformations. The combination of the above conditions also can cause the abnormal functioning of the pump. The most important

causes of CVI are the sequelae of the deep vein thrombosis and primary varicose disease.[14]

These are a few theories for the formation of venous ulcers.

Browse and Burnand's theory[15]

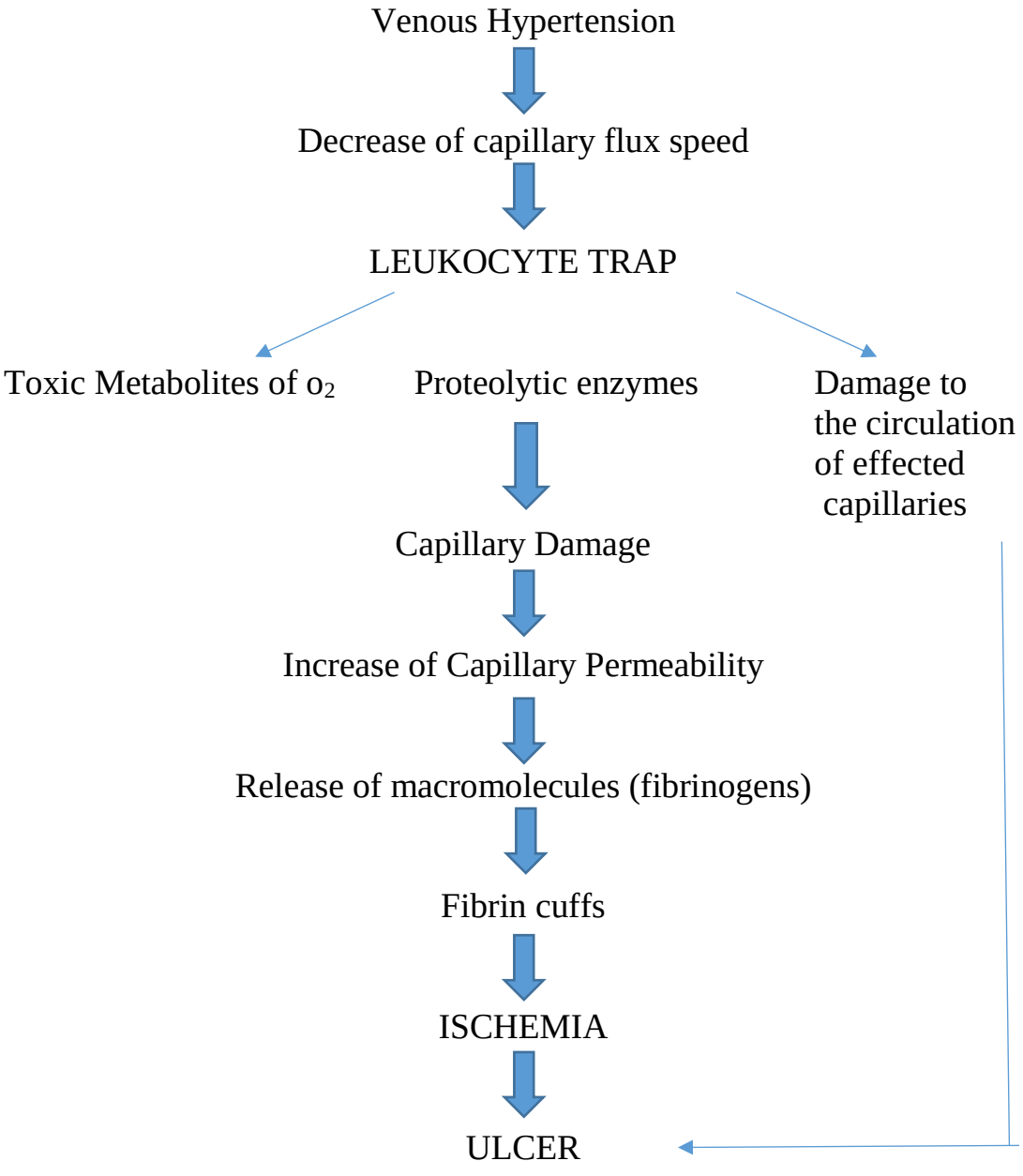


In the year 1982, Browse and Burnand proposed that the venous hypertension in the calf-muscle pump system is caused due to the transmission of blood to the superficial veins and the subcutaneous tissue of the calf region through the Perforating/communicating veins.

Due to this there is an increase in pressure which causes the dilatation of the capillary bed in that area and causes widening of the endothelial pores, which allows the passage of large molecules, like fibrinogen, to leak into the interstitial fluid.

As there is improper fibrinolysis in blood and interstitial fluid, this causes the formation of insoluble fibrin complexes. There is pronounced cell death and ulceration as the oxygen and other important nutrients are inhibited from reaching the cells due to the fibrin deposition around the capillaries. [15]

Theory of Coleridge et al.[16]

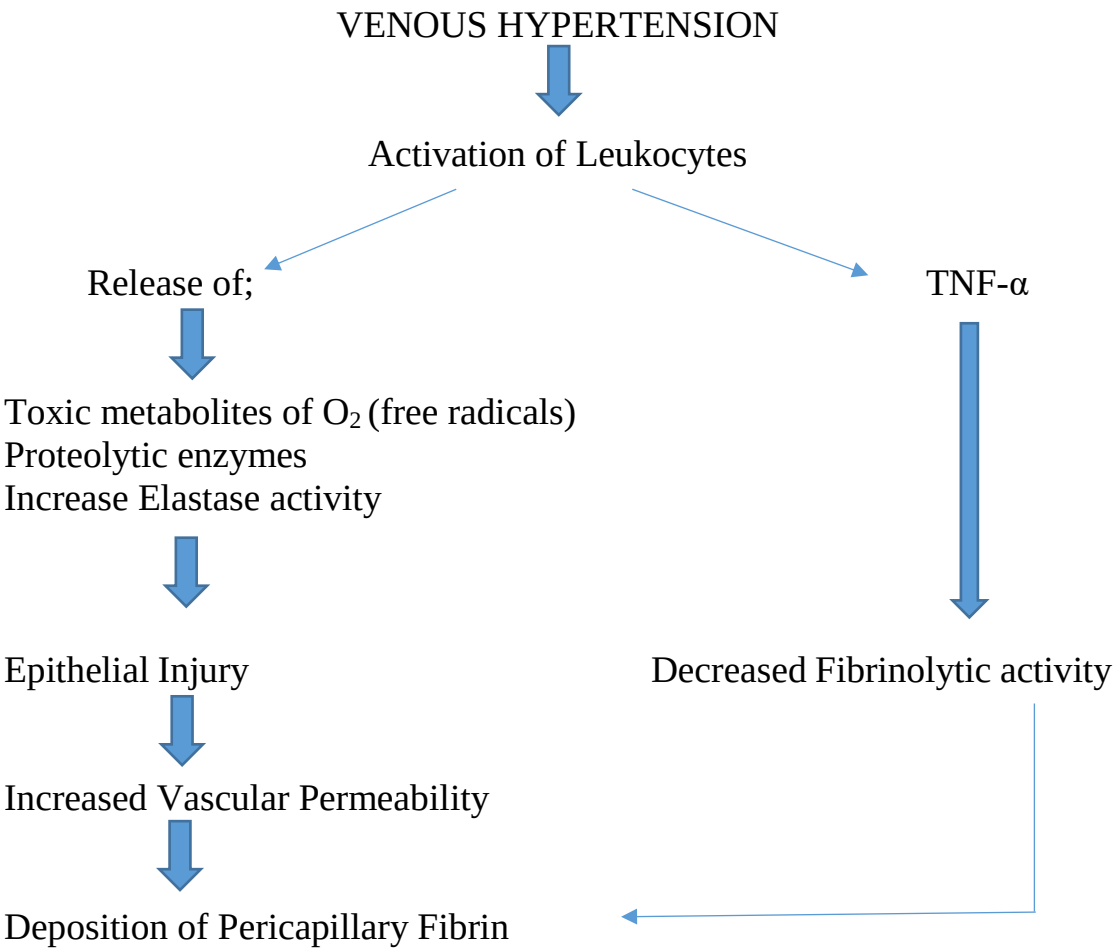


In the year 1988, Coleridge Smith et al. suggested a different hypothesis to the pathogenesis of the venous ulcer in a better way.[16] According to them, when a person is in the orthostatic position the pressure over the venous system increases, and this causes the pressure decrease in capillary perfusion, which in turn causes leukocyte trap as the capillary flux is significantly reduced.

Metabolites of proteolytic enzymes and oxygen are released from the trapped leukocytes which damages the capillary and makes it more permeable to larger molecules and resulting leukocyte trap. The capillaries leak fibrinogen and other plasma proteins as the permeability is increased which forms a fibrin cuff.

There is an area of ischemia around the affected capillaries due to the damage caused by the leukocytes that are trapped around the capillary loop.[16]

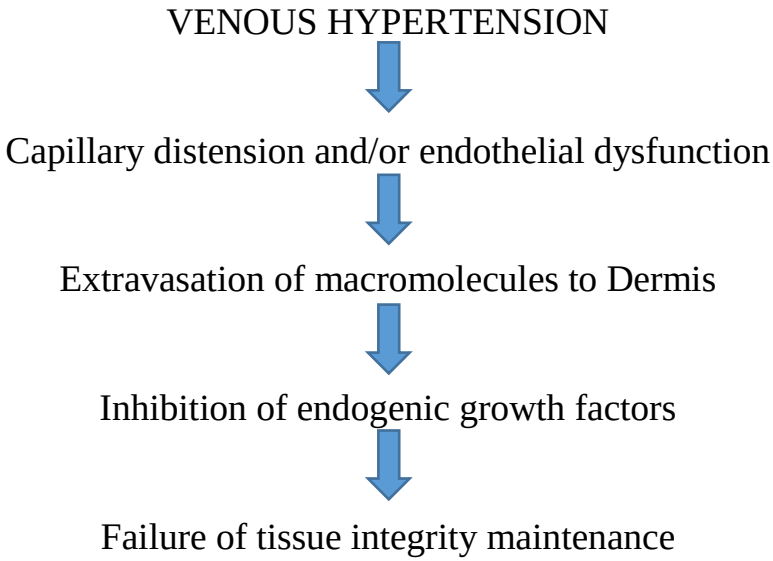
Theory of Claudy et al.[17]



In the year 1991, Claudy et al. suggested that free radicals and proteolytic enzymes are released from the leukocytes that are activated, and increased elastase activity, is responsible for epithelial injury and increases the vessel-wall permeability, causing the deposition of pericapillary fibrin. [17] They (leukocytes) also are a source of tumour

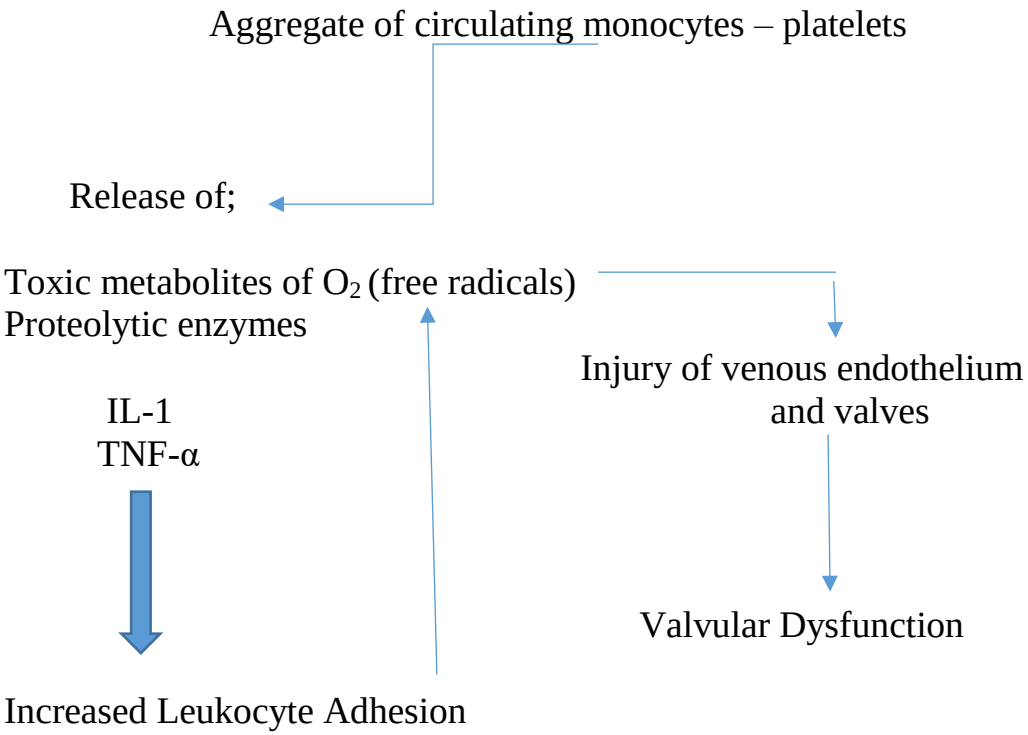
necrosis factor alpha (TNF- α), this reduces the fibrinolytic activity, and also caters to the production of pericapillary fibrin cuffs. These metabolites and fibrin released by leukocytes are the cause of delayed ulcer healing. [17]

Theory of Falanga and Eaglstein[18]



In the year 1993, Falanga et al. suggested that the leakage of fibrinogen, macromolecules and α 2-acroglobulins is caused due to the capillary dilatation or endothelial injury as a result of venous hypertension. The functioning of the endogenous growth factors is inhibited by these acromolecules, for example they inhibit the transforming growth factor β (TGF-β) from maintaining tissue integrity and healing. [18]

Theory of Powell et al.[19]



In the year 1999, Powell et al. proposed a relation among all subsets of CVI with exaggerated microcirculation levels.[19] They also proposed that valvular dysfunction could be caused by the damage done to the endothelium by the circulating monocyte and platelet aggregates. When the leukocytes are activated they release the above mentioned substances and when the platelets are activated they release interleukin-1 and TNF-α which cause the leukocyte to adhere.[19]

Clinical Features

It is very important for a physician to know in detail about this condition as early diagnosis and proper management can help in reducing the morbidity and the disability to a great extent, which if not done can be a cause of a serious psychological and socioeconomic burden on the patient and to some extent on their families.

Patients with venous ulcers are usually adults with a predominance to the female gender. They can present with the complaints of

a. Heaviness
b. Pain
c. Swollen limbs (increased while standing and reduces on elevation)

These ulcers are usually found around the medial aspect of leg i.e., Gaiter region or the medial malleolar area of the leg.

They can be either present as a single ulcer or could present with multiple ulcers having an irregular border, with sloping edges and shallow depth. Other changes like hyperpigmentation, lipodermatosclerosis, varicose veins oedema and stasis dermatitis may be present.

Diagnosis

The patients are usually diagnosed by the classic clinical features and other simple examinations. It is very important to examine and evaluate the patient thoroughly as a clinical diagnosis can be made on the basis of the following features

a. Wound with uneven margins
b. Usually superficial but can sometimes be deep
c. Well defined borders
d. The adjacent cutaneous erythema hyperpigmentation along with induration of various degree (lipo-dermato-sclerosis) can be appreciated
e. There can be some amount of exudate present, usually yellow in colour
f. Painless and
g. Usually around the medial malleolar area.

Other investigations such as

1. Doppler
2. Duplex scan
3. Plethysmography

Are also useful in detection of CVI.

Treatment

The two most important objectives to be kept in mind while treating a patient of venous ulcer are

1. To heal the ulcer as soon as possible and to ambulate the patient
2. To prevent the recurrences

Proper care of wound should be taken as it plays a pivotal role in healing. All the slough and necrotic tissue that is present on the floor of the ulcer should be removed surgically and the ulcer should be cleaned using saline. Care should be taken not to remove the granulation tissue that is formed on the floor of the ulcer. This step in very important as it minimises the chances of infection and promotes wound healing. After cleaning the ulcer / wound, the ulcer should be dressed in a moist occlusive dressing such as alginates dressing, hydrocolloid and hydrogel dressing so as to provide a humid micro-atmosphere which favours wound healing by controlling the exudates, encouraging epithelial cell migration, removal and prevention of any eschar and lysis of fibrin.[20, 21]

In a few instances when there is oozing and slough in the ulcer, it should be first treated with suitable antibiotics as healing is very difficult in the presence of infection. Pus culture and sensitivity can be done to treat

the ulcer with a suitable antibiotic. Sometimes biopsy from the ulcer also can be done for the same.

Treating the cause of the ulcer is of utmost importance such as treatment of varicose veins, incompetent valves and stasis by simple measures such as limb elevation and use of stockings can help to a very large extent. The use of stockings in the treatment of CVI is common worldwide but proper education of the patient about the manner in which the compression bandage or stockings are to be used to facilitate proper drainage of blood is important.

Other drugs that can be used as adjuvant therapy to compression dressings are aspirin and pentoxifylline which are shown to be effective.[22, 23, 24] But the efficacy of these drugs are still a topic of debate. Another study concluded that the administration of pentoxifylline in the dose of 800 mg 8th hourly along with compression bandage was effective in the treatment of venous ulcers. [25]

Another technique used in the treatment of these lesions is with the use of equivalent tissue-engineered skin. It has been proved to be very effective but requires specialized technology and is relatively expensive.[26, 27, 40, 56]

One of the major challenges is to avoid recurrences once the said ulcer is healed. Various studies have shown that about 30% of ulcers reoccur in the span of 12 months and around 75-78% reoccur in 24 months when proper care and treatment is not given. [28]

The patient must be counselled to use compression bandages lifelong so as to prevent new ulcers. The patient must also be explained to change the stocking in 3-6 months as they might lose their elasticity rendering the dressing ineffective.[29]

Wound Healing

Skin, being one of the 5 senses of the body, its function is not only touch recognition but also to protect the body by functioning as a protective barrier. Any major loss of integrity or continuation of this may lead to expose the vital structures present inside the body and could be a cause of morbidity and disability in the form of injury, infection and may occasionally even lead to death.

The most important objective for the treatment of the wound is to accomplish the wound closure as soon as possible so as to reduce the

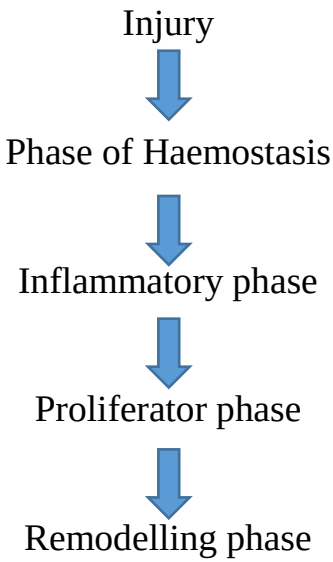
disability and morbidity of the patient. To do so with a cosmetically acceptable scar is the second objective in the treatment. As science has evolved greatly in the recent years it has given us more depth in understanding the complex cascade of wound healing and tissue remodelling. This understanding has helped us in improving the wound care that is required to reduce the healing downtime while achieving a functionally and cosmetically acceptable scar.

CASCADE OF TISSUE HEALING

Healing involves a balanced and an interactive process which deals with

1. Extracellular matrix
2. Parenchyma
3. Blood cells.

It includes four major steps



Phase of Haemostasis (the formation of a fibrin clot)

In almost all the wounds there is bleeding from the injured blood vessels. The formation of clot in at this time works as a protective barrier and simulates the function of the normal skin by shielding the denuded skin. It also functions as a bridge as it forms a complex matrix facilitating the migration of various cells in the repair phase.

This clot is made up of a mesh like framework of fibrin fibrils which is procured by the conversion of fibrinogen with the help of thrombin. This mesh is studded with platelets and also contains small quantities of thrombospondin, fibronectin and plasma fibronectin.

Another important function of the clot formation is that it caters as a stockpile of various cytokine and Growth Factor (GF) that are liberated in the degranulation phase of activated platelets. This primary mixture consisting the various cytokines and growth factors initiates and promotes the process of wound closure. It also promotes the recruitment of various circulating inflammatory cells to the site of injury. It also helps in the tissue migration essential of the re-epithelialisation, contraction of the connective tissue and promotes the neovascularisation required for healing.[30]

Through a large amount of varied chemotactic signals the neutrophils and monocytes are drawn towards the site of injury along with many other substances required for healing such as the growth factors which are released by the degranulation process of activated platelets and it also pulls in the formyl methionyl peptides which are derived from the cleaved bacterial proteins and other components of proteolysis of fibrin accompanied by matrix components.[31]

As a result of the molecular changes occurring over the surface of the endothelial cells lining the capillaries at the site of injury, the neutrophils and the monocytes circulating in the blood are recruited.

In the early phase the adhesion molecules of the selectin group form a light adhesion rapidly to slowdown the leucocytes and pull them away from the circulation of blood. In the next step the adhesion is tightened and it totally arrests the activated leukocytes which are pushed out between the endothelial cells into the perivascular space. The tightening of the adhesion is facilitated by b2 class of integrin's. [32]

Neutrophils usually start coming into the site of injury within a couple of minutes after the trauma. Earlier it was assumed that the only role played by the neutrophils was limited to remove the initial gush of infecting micro-organisms, but after a lot of studies it has been put forth that not only they help in inhibiting the bacterial growth and contamination but also are a place of origin for many pro inflammatory cytokines that provide the initial signals for the activation of the fibroblasts and keratinocytes that are present in the vicinity.[33]

If the site of injury is not infected, the purpose of neutrophils is served and they cease to exist in a few days of injury. Later they themselves get phagocytised with the help of tissue macrophages.

Macrophages still appear in the trauma site as they are recruited by the monocytes present in the blood as they are crucial for healing. If they are not recruited by the monocytes and are not present in the injured site then it impairs the healing in a significant way. [34]

Now the macrophages are entrusted with tasks such as phagocytosing any of the remnant bacteria that are harmful and clear the other debris. After they are activated they also help in healing by releasing a series of growth factors and

Cytokines ate the site of injury which help in promoting and amplify the signals released by the degranulated platelets and neutrophils earlier in the cascade.

The growth factors released by the activated macrophages are

1. Platelet derived GF
2. Vascular endothelial GF

These above mentioned growth factors play a vital role in initiating the granulation tissue formation. These cells with the help of the integrin receptor present on their surface bind to the specific proteins present in the

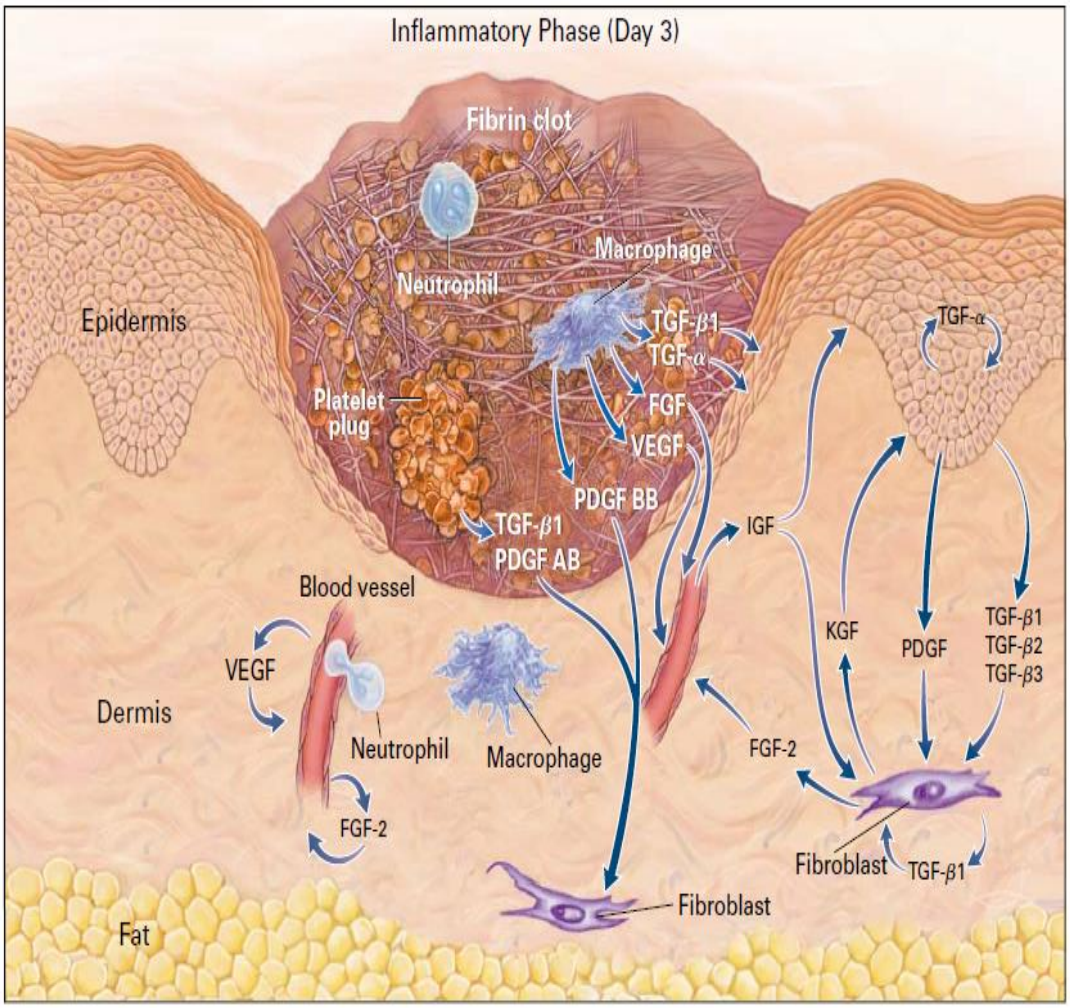
extracellular matrix and causes phagocytosis of the pathogenic micro-organisms and other fragments present in the wound.[35]

When the monocytes come in contact with the extracellular matrix they get metamorphosed and get converted into inflammatory or reparative macrophages.

The adherence to the extracellular matrix encourage the monocytes and macrophages to liberate

i. Interleukin- 1
ii. Tumour necrosis factor a (it is an important inflammatory cytokine)
iii. Transforming GF-a
iv. Platelet-derived GF (it plays a pivotal role in attracting fibroblasts)
v. Colony- stimulating factor 1 (It is required for the endurance of monocytes and macrophages)
vi. Transforming growth factor-b
vii. Insulin like growth factor I [36]

Hence monocytes and macrophages are very much essential for healing as they release various growth factors which are required for proper and effective healing. It has also been shown that wound healing is difficult and defective in animals who are deficient in macrophages. [37, 38]



Growth factors responsible for healing are shown in the image above.

Epithelialization

This is one of the most important phases of wound healing. It can start as soon as a few hours after the injury.

The adnexal structures such as the hair follicle have epidermal cells which shrug off the clotted blood and stroma from the tissue damage site. Around the same moment, there is significant phenotypic alteration which

includes the pulling back of tonofillaments. The intercellular desmosomes dissolve and acts like a bridge allowing the cell migration. [40, 41]

As the hemidesmosomes dissolves the connection between the epidermal and dermal cells is lost, facilitating the lateral movement of the cells.

The eschar is separated from the living tissue by the migration of the epidermal cells. This level of separation is decided by the array of integrin that the cells express on the membranes.

The collagen and extracellular matrix proteins is degraded by the collagenase which is activated by the plasminogen activator. After around one or two days post injury the cells start migrating actively which is followed by the epidermal cells which are present at the wound margin.

But which stimuli causes the induction of this response is still not clear. One of the stimuli that causes this is proposed to be the liberation of growth factors and pronounced expression of its receptors promotes this processes. Other noteworthy proposals include the

i. Keratinocyte GF
ii. Transforming GF
iii. Epidermal GF [42-44]

The basement membrane proteins start appearing again in an orderly fashion starting at the margin of the wound and travelling towards the centre of the wound like the way a zip works. All this starts after the re-epithelization starts.[45]

Once this whole process is completed the epidermal cells return to their normal physical appearance and are attached tightly once again, to the newly formed basement membrane and to the dermis lying below it.

Formation of Granulation Tissue

The newly formed granulation tissue also called as the matrix, starts developing in the injury site at around 96 hours from the infliction of trauma.

The newly formed matrix along with its granular appearance contains multiple newly formed blood vessels. The new capillaries along with the fibroblasts and macrophages enter into the injury site around the same moment.[46]

There is continuous supply of the necessary growth factors by the macrophages which promote the development of new vessels and the production of new fibrous tissue.

The newly formed extracellular matrix that is essential for the re-enforcement of the ingrowing cell population is provided by the fibroblasts and the supporting nutrients and the oxygen required by the cell to maintain its metabolism is supplied by the newly formed blood vessels.

The fibroblasts present I the vicinity around the trauma site are induced to release the required integrin receptors, increase in number and to move into the trauma site by the growth factors which are

- Transforming GF β 1
- Platelet-derived GF

Both of which act in coordination with the extracellular-matrix molecules.[47, 48, 49, 50]

Many studies show the efficacy of the platelet derived growth factor in treating the trophic ulcers and diabetic sores [51, 52]

They have also shown the efficacy of the basic fibroblast growth factor in the treatment of chronic trophic ulcers.[53]

The development of granulation tissue is facilitated by the provisional matrixes which is also called as the basic fundamental molecular units of the freshly established extracellular matrix by providing a bridge or a scaffold essential for the cell to migrate[46]. The most important step is the production of fibronectin and the appearance of specific integrin receptor which is bound to either fibronectin or to the fibrin or to both and facilitate the production of granulation tissue. [50, 54]

The production, aggregation and re-modelling of the extracellular matrix is caused by the fibroblasts. On the contrary there can be a positive or a negative influence on the potential of the fibroblast for the same by the extracellular matrix.[50, 55]

A potent proteolytic substance is required by the cell to form a path required during cell migration into the tightly packed extracellular matrixes or into the cross-linkages of the fibrin in the blood clots.

There are many such enzymes that are produced by the fibroblasts that are suitable for this task such as

- Gelatinase A
- Serum-derived plasmin
- Stromelysin
- Plasminogen activator
- Collagenases.[56,57]

The extracellular matrixes are synthesised with the help of fibroblasts when it enters into the injury site. At this time the old extracellular matrixes are removed and in their place there is the production of new collagenous matrixes.[55, 58] This could be due to the transforming growth factor β1[55.]

Fibroblasts stop the production of collagen after the wound has received surplus amounts of collagen matrixes, after which there is scar formation which is the stage where the fibroblast granulation tissue is removed. A few signals of which we know less trigger the apoptosis of the cells present in the trauma site.

Neovascularization

It is the step where there is formation of new blood vessels for the newly produced cells. It is of utmost importance to support the granulation tissue which has just been formed. Angiogenesis is a complicated cascade that depends on the extracellular matrixes present in the trauma site and also on the stimuli from the endothelial cell and the cell-migration.[59]

In the earlier days it was presumed that only the acidic or basic fibroblast growth factor was responsible for the production of new blood vessels. But later it was discovered that many other substances have the potential to stimulate the production of new blood vessels. Some of them are

i. Angiogenin
ii. Fibroblast growth factor
iii. Angiopoietin 1
iv. Thrombospondin
v. Angiotropin
vi. Vascular endothelial growth factor
vii. Transforming growth factor β [60-62]

Stimulation of neovascularization can also sometimes occur by the

viii. Elevated lactic acid
ix. Low oxygen tension [63]

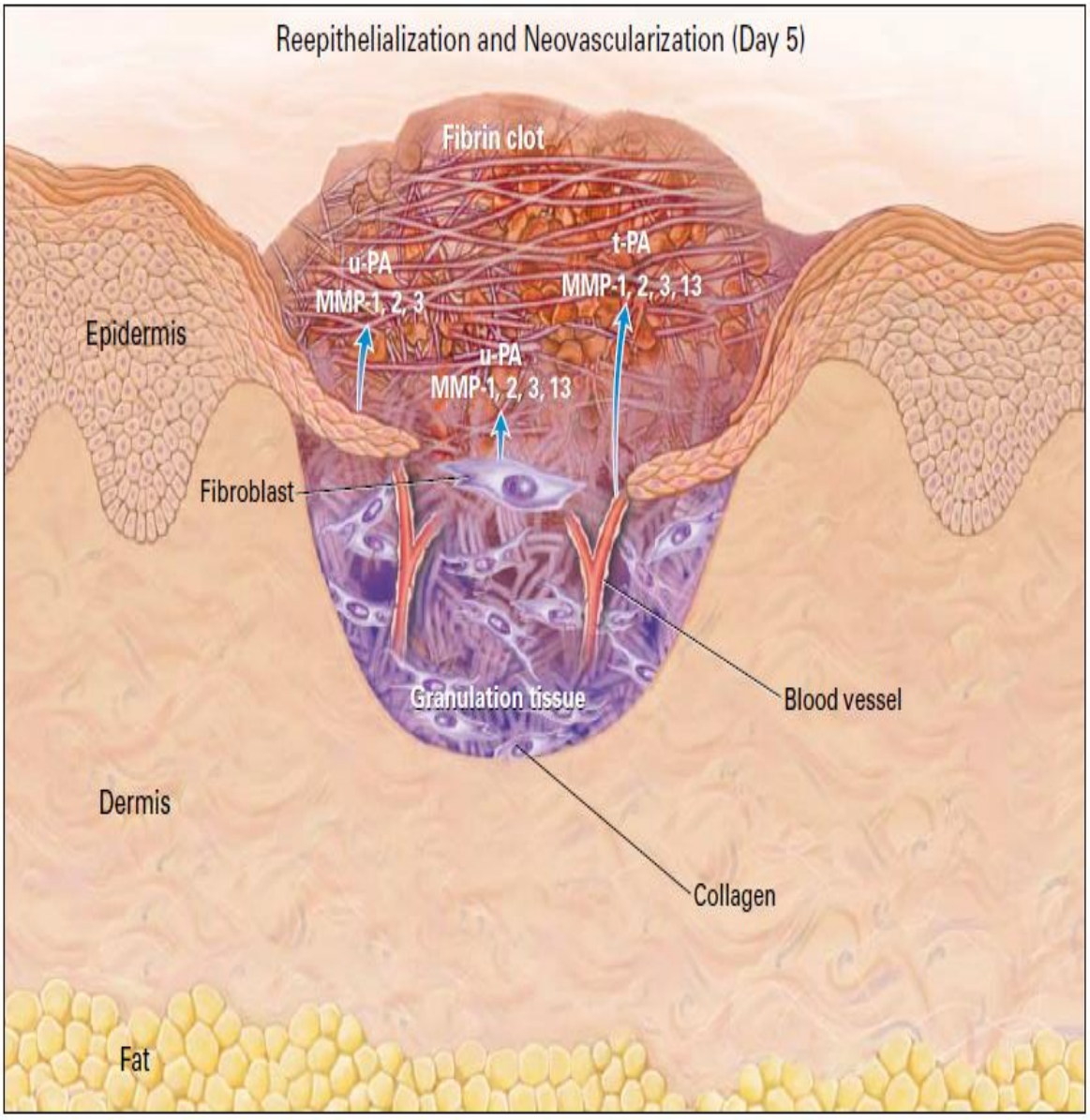
Most of the above mentioned substances promote the liberation of vascular endothelial GF or the fibroblast GF by the endothelial cells and macrophages to induce the production of new blood vessels.

In the first two to three days of tissue healing fibroblast growth factor may be the key in the development of new vessels but when the production of blood vessels during the development of granulation tissue is concerned then the vascular endothelial- cell growth factor plays the most crucial role.[64]

Not only the above mentioned factors but in addition to that proper extracellular matrixes and specific endothelial receptor are required for the production of new blood vessels. The endothelial cells require a bridge to migrate into the trauma site which is provided by the perivascular fibronectin. This fibronectin is derived by the rapidly multiplying microvascular endothelial cells which are present in the wound area and in the vicinity which temporarily deposit large quantities of fibronectin in the vessel wall. [65]

Trauma of the tissue causes degradation and reduced oxygen supply. The macrophages promptly produced fibroblast growth factor after the cell destruction and due to reduced oxygen levels epidermal cells produce vascular endothelial-cell growth factor. At this point the extracellular

matricidal proteins are degraded by the proteolytic substances which are present in the connective tissue. This also pulls the monocytes from the blood and brings them into the injury site where it is converted into activated macrophages and this produce angiogenesis factors.



As the basement membrane undergoes fragmentation the endothelial cells which are induced by the above mentioned factors undergo migration

and produce new blood vessels in the wound. After the trauma site has received sufficient amounts of new granulation tissue the production of new blood vessels stops and any unwanted or extra blood vessels present will undergo apoptosis. [67]

Wound Contraction and Extracellular-Matrix Reorganization

Contraction or the closure of the wound consists of a complicated yet beautifully choreographed interactions between the cytokines, extracellular matrixes and the cells present at the site. Multiple bunches of actin containing microfilaments are present in the cytoplasmic side of the plasma-membrane, these look like myofibroblasts but basically are fibroblasts and are seen from 7^{th} -14^{th} day of healing. [58, 68]

The contraction of the injury site is assumed to have started when the myofibroblasts appear in the wound. The contraction of the wound.[69] is caused by the following

i. Attachment of fibroblast to the collagen matrixes [71]
ii. Cross-linkages among individual bundles of collagen. [72]
iii. Transforming GF β1 or β2
iv. Platelet derived GF preparation of [70]

Collagen remodelling is based on the persistent production and catabolism of collagen at a slow pace at the time of conversion from the granulation tissue to the scar-tissue. Matrix metalloproteinases which are the proteolytic enzymes regulate the destruction/degradation of collagen in the trauma site. These metalloproteinases are secreted by many cells, such as

i. Epidermal cells
ii. Fibroblasts
iii. Macrophages
iv. Endothelial cells [56]

Tissue inhibitors of metalloproteinases and matrix metalloproteinases play a major role in the wound contraction and healing.[73]

The remodelling occurs by the contraction of trauma site rapidly in the first three weeks as there is rapid filling of the wound area by the fibrillary collagen. At this time the scarred skin attains only around 20 % of the actual strength it can attain later in life. After this step the scarred skin increases its tensile strength but the rate at which it increases is very slow when compared to the first few weeks of healing. This shows that the

rate at which the collagen gets accumulated becomes much slower after a few weeks of healing.

However the scarred skin can never get the tensile strength equivalent to the normal uninjured skin. The maximum strength that it can attain is 70% as that of normal uninjured skin.[74]

GROWTH FACTORS IN WOUND HEALING

Epidermal Growth Factor And Transforming Growth Factor-Alpha

There are multiple GFs that have been brought to light by man. All the growth factors play a crucial role in healing in their own special way. Out of all these factors Urogastrone is the most popular/well known growth factor which is also known as the Epidermal Growth Factor (EGF). It was first discovered in mice in the year 1962. It was extracted from the submaxillary gland of the mice.

It is a small polypeptide of molecular weight of 6000Da and consists of 53 amino acids. It is similar to the TGF-α which is a 50 amino acid polypeptide. It shares about 42% of the properties of TGF-α. Due to this similarity it is also functionally similar to TGF-α [75]

As they are similar they also share the same receptor which is found in abundance in the skin, its adnexae and the GI tract. [76]

It was shown that in pigs EGF speeds up the process of healing after semi thickness burns, and also in the humans who have donated skin grafts of partial thickness.[77, 78]

Different types of cells are stimulated by the EGF. They are

- TGF-α which are present in the platelets
- Muscle cells and fibroblasts.

TGF-alpha which is present in the platelets are liberated when they come in contact with the injured tissue. Few studies have also shown that in human beings and animals it helps by promoting corneal epithelial regeneration[79] Which also helps in increasing the tensile strength in patients with wounds due to incision and site of partial thickness skin grafts.[78]

TGF-α protein is present in very high quantities in the epidermis of the hyperproliferated psoriatic skin. Even though it is not generated in the keratinocytes, its receptor is still present in the normal skin. It is also known to play a major role in the regulation of the normal growth of skin and its differentiation. It may also be useful in the treatment of gastric ulcers as it has been shown to stimulate gastric mucosal growth when injected intravenously in rats.[80]

Insulin-like growth factor-I

This is another important substance which plays a crucial role in the wound healing. Insulin-like growth factor-I (IGF-I) is a polypeptide weighing 7650 da and is made up of 70 amino acids. It is also called as somatomedin-C[81] and on the structural front it shares its properties with proinsulin. This is generated primarily in the liver, but it is also produced in the cartilage and skeletal muscle on which the growth hormones have an anabolic effect. It is also known to increase the efficacy of the growth hormones in the infancy period. [82] Growth hormone is known to play a role in the regulation of generation of IGF-I. But it is also known that there is a growth hormone independent production unit of IFG-I which produces this without the help of growth hormone.[83, 84]

Liver is the most important source of IGF-I as it is the major producer of this factor in the human body. This makes it unique from other growth factors as it also has an endocrine effect along with the usual autocrine and paracrine action.

IGF-I generation is significantly increased in the multiple types of tissue that are in the regenerating phase like the peripheral nerves and the skeletal muscle [85] In the granulation tissue there is more amount of IGF-I messenger RNA[86,87] and protein.

Though there is very less supportive theory of IGF-I stimulating wound healing. Treatment with this alone showed no improvement in the wound size in the pigs, but it is thought that it can play a role by stimulating PDGF which intern plays a major role in the healing and dermal generation.

Some studies state that it has not got any stimulating result on the mononuclear cells in the trauma site.[88, 89] In some other studies it has been concluded that the wounds in rats which was injected with IGF-I after hypophysectomy had increased no of macrophages when compared with control group. In another study that was carried out it was shown that in the wounds which had damaged healing due to prior treatment of steroids, IGF-I injection improved the healing in those cases.[90]

To summarise IGF-I does not have a major role in the wound healing directly, but it can stimulate the same as it acts as an autocrine growth factor and also promotes the manufacturing of collagen from the cultured fibroblasts [91]

Insulin like growth factor II

Insulin like growth factor II is similar to the above discussed IGF-I. It is about 50% similar in the structural aspect to the IGF I.[92] This is made up of 67 amino acids.

It holds a crucial role in the maturation of the neonates. This is probably the reason that during the development of the baby in the uterus the insulin like growth factor is present in abundance when compared to the IGF-I. but this is not the case after birth where the IGF-I increases as the production of Insulin Like Growth Factor II goes down in all most all the parts of the body. Brain is the only organ that even after birth still produces the Insulin like Growth Factor II.[93]

The wound healing capabilities of the Insulin like Growth Factor II has not been fully studied. [94] But it may help in healing by interacting with the type I receptor which may cause cell growth. However its main affinity lies with the type II receptor.

Basic Fibroblast Growth Factor

Basic Fibroblast Growth Factor (bFGF) is one of the most important growth factors discovered. Its molecular weight is about 17000 Da. It is consists of a single chain of 146 amino-acids. It belongs to the family of proteins that are related and acidic fibroblast growth factor is one of the most popular growth factor. [95]

Basic fibroblast growth factor has its effect on most of the cells of the body.

Few of them which play an important role in the healing cascade induced by the basic fibroblast growth factor are

1. Smooth cells
2. Astrocytes
3. Peripheral nerves [96]
4. Multiple tumour cells
5. Adrenocortical cells
6. Vascular endothelial cells
7. Fibroblasts (Most importantly)

Basic Fibroblast Growth Factor is known to be one of the most important growth factors as it not only has its effect on the migration of the endothelial cells, it also plays a role in the formation of new blood vessels. Multiple animal models have suggested that it has angiogenesis induction capabilities.[97]

In a few studies it has been shown that along with the strong mitogen capabilities it also helps in the production of blood vessels indirectly in vivo by causing inflammational changes.[98]

Many studies have shown the ability of bFGF to accumulate the macrophages, capillaries and fibroblasts in the wounds which are either incisional or are subcutaneous chambers. [99,100] Another study in a similar model showed the reduction/inhibition in the granulation tissue formation when the antiserum for basic fibroblast growth factor is injected.[101]

It has been proved that the concentration of bFGF mRNA is much more in the granulation tissue when compared to the normal skin fibroblasts.[102, 94] In a study it has also been shown that in animals like mice which were obese, diabetic or which were treated with steroids and the wound healing was slow, the administration of bFGF caused speeding up of healing and repair in the full thickness skin wounds.[104]

In another study which had pigs as the subjects bFGF did not show much improvement in wound healing in the tested concentrations. But it showed much more significant results when it was combined with TGF-α or IGF-I by showing elevation in the concentration of hydroxyproline [105]

bFGF is a strong promoter of cartilage repair as it influences the growth of chondrocytes and the formation of the extracellular matrix. [106] It also has great affinity to the heparin and probably that's the reason that it binds to heparin sulphate proteoglycans and can be consumed as required.

Platelet Derived Growth Factor

This GF is a large molecule which is made up of more than 100 amino acids. It has a dimeric structure which comprises of A chain and a B chain. Both the chains have more than 100 amino acids. This factor is present in three forms

- AA
- BB
- AB

All these three isoforms have a different mode of action.

PGDF is derived from all the below mentioned cells

a. Fibroblasts
b. Macrophages
c. Endothelial cells
d. Platelets (largest source)

All the above mentioned cells take active participation in the wound healing.[107]

This factor is the one that reaches the site of injury as it is produced from the granules of platelets and this is the major producer of PGDF in the body.

It attracts the neutrophils and macrophages and promotes the migration of fibroblast as these cells are very important in the process of wound healing.[108]

By the addition of PGDF into the wound causes marked increase in tissue deposition.[109]

Some studies have also shown that in animals which have diabetes and because of which the rate of improvement is reduced, in such animals injecting PGDF brings the rate of healing to normal.[110]

It is also promotes healing in full thickness wounds in rabbits and it is also a strong promoter of the production of granulation tissue in rats. All this is caused by the accumulation of glycosaminoglycans and inflow of the extracellular macrophages.

In a study multiple growth factors were compared and the combination of PGDF and TGF-α and PGDF and IGF-I were considered to be the most significant.[106] A study in humans which was double blinded and placebo controlled was also conducted in which PGDF was used to treat pressure ulcers and it showed to help in reducing the size of the ulcer by the end of 28 days when compared to the control group.[112]

Transforming growth factor β

This is also a very important growth factor which plays a crucial role in the wound healing. This is also a dimer like the platelet derived growth factor. Each chain consists around 112 amino acids.

Genetically five different types are present type 1, type 2, type 3, type 4 and type 5. Out of all these TGF β is the most widely popular. It promotes the multiplication of connective tissue and a few other cells. It also limits the multiplication of most epithelial cells and lymphocytes.[113]

It is liberated by the wound fluid, macrophages and the platelets. It is known to promote healing in the subcutaneous wound pockets in animals and also in the incisional wounds.[114, 115,116] it is also concluded that it was able to invert the effects of delayed healing caused by glucocorticoids. [117]

There are a few studies which suggest that it plays a role in the scar formation. When compared to other growth factors it is also able to enhance significantly the contractibility of the fibroblasts present on the collagen matrixes. [118]

In a few more similar studies it has been shown to play a role in the late stage of healing. It is also shown to specifically promote the mature collagen bundles when compared to any other growth factor.

Hence it is concluded that it specifically promotes the initiation and maturation of collagen in the starting stages of healing.[119]

Tumour necrosis factor-α

Tumour necrosis factor α is produced by the macrophage cells.[120] It is a cytokine which limits the metastases and suppresses the tumour formation in mice. [121] It has been proved that it plays an important role in the evolution of septic shock. When present in low quantities even then it have a significant effect of regulation on the neutrophils.[122]

In rabbits it causes the production of new blood vessels in the corneas and it also improves the growth.[123] Many other investigators suggested that the above mentioned effects was due to the promotion of inflammation and that it actually suppresses the growth of endothelial cells.

It was shown that it had reduced expression of type 1 collagen and wound-bursting strength in the wounds in rats. It has also been proved that it decreased the fracture healing capacity in rats.

Hence it is concluded that it is not a factor that greatly improves healing, but it plays a very small part.

Growth Hormone

Growth Hormone (GH) is a hormone that is produced by the anterior pituitary gland. It is also called as somatotropin and it made up of 191 amino acid peptides. This hormone is of utmost importance in the proper growth. There are 190 amino acids peptides in the rats GH and is 66% similar to that of a human being.[124]

It was proved in different studies that GH has a role in the normal growth and it enhanced growth in the rats which were injected with GH.[124, 125] GH has a direct effect on the liver and it encourages the production of IGF-I which helps in growth. There was increased production of IGF-I and unilateral growth of the tibial growth plate when the injections of GH were given locally into the tibial growth plate.

It also helps in attaining an anabolic effect by increasing the number of IGF-I and IGF-mRNA in multiple target organs. Other effects like the body growth is carried out by the locally available IGF-I which produces the autocrine and paracrine effect. The above process is initiated and promoted by the growth hormone.[126]

Receptors for the growth hormone are present in the following

a. Fibroblasts
b. Chondrocytes
c. Skin
d. Skeletal muscles
e. Adipocytes

These receptors have 638 amino acid peptides and is present as a single chain. The extracellular part of the receptor and the binding protein of the GH are identical.

GH also plays a role in the metabolism of the following

- Fluid
- Minerals
- Lipids and
- Carbohydrates
- It also plays a role in the production of proteins

GH is known to play a role in reducing the time taken for healing and by increasing the quality of healing by producing an anabolic state and also by the exaggerated protein production.

The main basis of applying the growth hormone in wounds is that at least one of the type of cells present in the wound will have the growth hormone receptor and this is not only the basis but is also a prerequisite for this procedure.

Multiple studies have shown that the growth hormone receptors are present on the fibroblasts which are present in the dermis and on the epithelial cells in the epidermis.[127] Both the GH and IGF-I play a role in the collagen gene expression and in the extracellular matrixes production in fibroblast culture.

In other studies it proved to be beneficial in reversing the reduction of growth caused by starving and had increased the mechanical strength of the incisional wounds when administered systemically in rats.[128, 129]

It was proved that IGF-I Plays a mediating role in the impact of GH on healing as in a study when GH was used as a local application there were exaggerated quantities of IGF-I mRNA present in the wound and there was also a 50 % shoot up of granulation tissue.[103,111]

In a study it was shown that when there was no GH it resulted in less amounts of IGF-I, hydroxyproline, DNA and granulation tissue. Hence this proves that it is necessary to have GH for proper and fast healing.[130,131]

Platelet Rich Fibrin in the Treatment Of Chronic Ulcers

All wounds which are less than 6 weeks old are called as acute wounds and they heal by a systematic but complicated cascade of molecular and physiologic procedure. The cascade includes many steps which are discussed in detail above. But healing is not the same in the wounds which are older than 6 weeks and these wounds are called as chronic wounds [1]. When compared to the acute wounds the healing process in chronic wounds appears to be stuck somewhere between multiple local and systemic factors such as

a. Severe bacterial infection
b. Abundant necrotic tissue
c. Lack of blood supply
d. Reduced response to growth factor and decreased production of the same
e. Lack of responsiveness to the cell signals [132]
f. Venous insufficiency

To treat such chronic ulcers they must be first cleaned and proper debridement must be done, followed by ideal dressing which can limit the growth of pathogenic bacteria and also provide a moist micro-environment that is necessary for healing.

But even when all this is performed in the appropriate technique, still in a few instances there is no improvement in healing and here comes in advanced therapeutic options such as

i. Complimentary therapy
    - Electrical stimulation
    - Ultrasound
    - Negative pressure
    - Hyperbaric oxygen
ii. Dermal matrix equivalents
iii. Cellular therapies to replace deficient components
    - Living skin equivalents
    - Allografts,
    - Autologous epidermis
iv. exogenous growth factors
    - Growth factor/ fibrin preparations

- Autologous growth factor
- Purified single growth factors [132]

Products obtained from blood which were used to seal and heal wounds and ulcers started around 5 decades back[142]. For ulcers the platelet concentrate that was used was the Platelet Rich Fibrin.

The effectiveness of products obtained from blood in wound healing was first described by Whiteman et al.[141] following which the use of these products became extremely popular in the last 15 years.

There are multiple platelet rich concentrates that are available in the market and each type of concentrate has its own applications in medicine. These concentrates depend on the amount of fibrin and leucocyte present in them. They are

i. Pure platelet rich fibrin
ii. Leucocyte and platelet rich fibrin
iii. Platelet rich plasma
iv. Leucocyte and platelet rich plasma.

These are available as readily available products that be used directly. However products prepared from the patient's own blood is advised as this reduces the chance of unnecessary infections.

Preparation of platelet rich fibrin is a process where the whole blood is processed and the resultant material produced consists 60 times more fibrin and platelets when compared to the normal human blood. The resultant material is a jelly like substance which is pale yellow to yellow in colour.

Wound healing usually remains the same no matter for which tissue it is. It includes many steps in a sequential manner comprising of humoral and cellular parts which also involves the inflow of platelets and its activation. [133]

The successive production of growth factor and cytokine furnish the primary impetus for the healing procedure to begin. The platelets then liberate multiple growth factors such as the

- Transforming GF
- Platelet-derived GF
- Vascular-endothelial GF
- Platelet derived epidermal GF

- Insulin like GF-I
- Basic fibroblast GF

It is also proved that when applied locally on the wound it helps in wound healing which has been the principle of using Platelet Rich Plasma (PRP) for the past many years. [134-138]

Preparation of autologous PRP

This process consists of the collection of blood from the patient in aseptic conditions and separating the plasma and the platelets from the other cells that are present in the whole blood. After this the next step is the polymerization of the obtained fibrin that is present in the plasma, so that the platelets are now concentrated in the form of a jelly which is rich in platelets.

As it is in the form of a jelly it gives us that stability that is required to use it as a dressing in the wounds. As of today multiple commercial procedures are present to obtain PRP by using thrombin and calcium. This is used to make the desired platelet rich fibrin matrix.

To make this fibrin matrix extra whole blood from the patient is required when compared to the blood required for the production of PRP. The advantage with using thrombin is that it causes the instant liberation of the desired growth factors.[139]

The other method is by just using calcium and centrifuging the whole bold to derive the platelet rich fibrin matrix. In this method the addition of thrombin is avoided. The process of centrifugation causes the activation of the polymerised fibrin to produce a Platelet Rich Fibrin Membrane (PRFM). This is the platelet rich jelly which can be in the form of a gel or a membrane and both of which can be easily applied on the wound using a pair of sterile forceps. At this point the autologous activators that are situated in the trauma site precipitate the liberation of platelet-derived growth factors. Using this procedure it is ensured that there would be slow release of the required growth factors in the trauma site. This growth factor that is released attracts various cells required for wound healing from time to time which then helps is the healing cascade.

Multiple studies have shown that with the use of PRFM there can be slow and sustained release of the required growth factors continuously till up to as long as 7 days.[140]

It (PRFM) acts as a stockpile of the necessary growth factors that are required for the timely healing of any wound. It also provides the required fibrin bridge required for the tissue migration required to help in the healing procedure. Hence this also provides a very easily acceptable dressing regimen where the dressing is changed once in a week so as to maintain the continuous supply of the growth factors that are required by the wound for the prompt and steady healing.

One of the most common cause of ulcers in the lower limb in old age is the venous ulcer. This is usually seen in the adult population, and due to the nature of the disease, the re-occurrence of the venous ulcer is common. This becomes a matter of great concern for the patient as it becomes the cause of great psychological and socio-economic burden. It also becomes the cause of disability and morbidity which has a significant impact on the work-days lost. This in-turn along with the expenditure for treatment of recurrent ulcers becomes the cause for the socio-economic burden not only for the patient but for the patient's family as well.

One of the main causes for the development of venous ulcers is the lack of supply of blood and nutrients due to the stasis and fibrin cuff formation limiting the supply of oxygen and other necessary nutrients for the proper maintenance of the skin.

In such patients when other treatments fail, the use of PRF is justified as it supplies all the growth factors and nutrients that are necessary for the healing of chronic venous ulcers.

MATERIALS AND METHODS

STUDY DESIGN

- Open label prospective randomized control trial

STUDY PERIOD

- This study was conducted for a duration of one year in patients suffering with chronic venous leg ulcers.
- This study was conducted only after receiving the acceptance of the ethical committee.

STUDY POPULATION

- All the patients who attended the OPD of Dermatology Venereology and Leprosy and were suffering from chronic venous ulcers became a part of the study.

INCLUSION CRITERIA

- Patients suffering with chronic venous ulcers of lower extremity for more than 6 months, attending the OPD of Dermatology Venereology and Leprosy.
- Having an ulcer area of 1cm x 1cm to 5cm x 5 cm.

EXCLUSION CRITERIA

1. Ulcers of less than 6 months duration.
2. Ulcers of other etiology such as
    - Neuropathic ulcer
    - Arterial ulcer
    - Diabetic ulcer
    - Ulcer with underlying vasculitis
3. Patients with osteomyelitis affecting the area of the ulcer
4. Ulcers with exposed tendons or bones
5. Ulcers with area <1cm x 1cm or > 5cm x 5cm
6. Ulcers with copious discharge /overt infection /infected with Pseudomonas
7. Patients receiving anti-coagulants/ anti-platelet drugs/ bleeding diathesis.
8. Patients with age < 18 years or > 80 years
9. Pregnancy and lactation
10. Non-consenting patients

METHODOLOGY

No. Of Groups Included In The Studied: 2 groups

- Group 1 (Treatment Group): Patients with chronic venous ulcer receiving Platelet Rich Fibrin (PRF) dressing along with standardized regimen of good wound care and rest.

- Group 2 (Control Group): Patients with chronic venous ulcer receiving moist saline dressings along with standardized regimen of good wound care and rest.

PREPARATION OF PLATELET-RICH FIBRIN (PRF)

Prerequisites for the Preparation of PRF:

i. 10 ml disposable syringe (Figure 1)
ii. Sterilised vacutainer (Figure 1)
iii. Sterilised non-toothed forceps (Figure 1)
iv. Sterilised kidney tray (Figure 1)
v. 11 number blade (Figure 1)
vi. Roller bandage (Figure 1)
vii. Sterilised gauze pieces (Figure 1)
viii. Sterilised gauze pad (Figure 1)
ix. Centrifuge machine (Figure 2)

Procedure

Patients is explained about the procedure in detail in the local language and the patients consent is obtained. The patient is made to lie down comfortably and 10 ml of the patients own blood is withdrawn with the help of a 10 ml disposable syringe from the brachial vein. The 10 ml of blood that is obtained from the patients is immediately transferred into a sterile vacutainer. This vacutainer is then placed in the centrifuge machine with another vacutainer containing water which acts at counter weight on the opposite side. This is then centrifuged at 3000 rotations per minute for 15 mins. After 15 minutes a fibrin gel is acquired in the centre of the vacutainer, in the middle of the RBCs which are settled at the base and acellular plasma above. 10 ml of whole blood will yield about 2.5 ml of clot.

Dressing Procedure

Group 1: Patients is explained about the procedure in detail. The patient is made to lie down comfortably and 10 ml of the patients own blood is withdrawn for the production of PRF. The ulcer is cleaned with a sterile gauze and saline and the measurements of the ulcer are taken. After 15 minutes the vacutainer is removed from the centrifuge machine without

shaking the vacutainer much. Using a sterilised non-toothed forceps the platelet rich fibrin gel that is obtained after the centrifugation is removed carefully and is placed on a sterile gauze piece which is already placed in the sterilised kidney tray. The gauze piece is soaked in saline before placing the PRF gel on it. With the help of a 11 number blade, the RBC clot that is adhering to the PRF gel is scraped off. The PRF gel is then placed on the ulcer floor and is covered with a sterile gauze (primary dressing), which is in turn covered with a sterile gauze pad (secondary dressing) and is covered with a sterile roller bandage. This dressing will be left in place for a period of one week. After 1 week, all PRF remnants will be removed with water and sterile gauze. Following this, the next PRF treatment was given. A total of four PRF treatments at weekly intervals were given for a total duration of 4 weeks.

Group 2: Patients is explained about the procedure in detail. The patient is made to lie down comfortably. The ulcer is cleaned with a sterile gauze and saline and the measurement of the ulcer is taken. The ulcer is covered with a sterile gauze soaked in saline (primary dressing), which is in turn covered with a sterile gauze pad (secondary dressing) and is covered with a sterile roller bandage. This dressing was left in place for a period of one week. After 1 week, slough (if any) will be removed with water and sterile gauze. Following this, the next saline dressing was done. A total of

four saline dressings at weekly intervals were done for a total duration of 4 weeks.

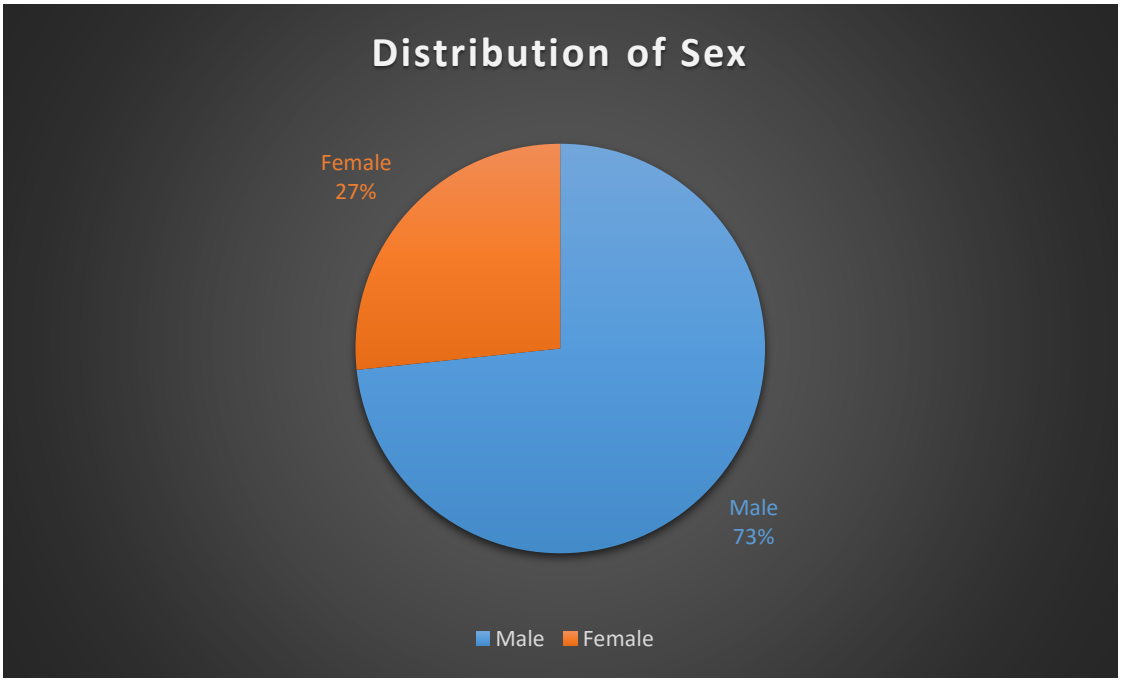
Measurements Documentation

- The greatest length and the greatest breadth were measured using a thread and a scale. This was done before starting the treatment, before repeating the treatment each time at weekly intervals and after the treatment was completed (the final measurement).
- Digital photographs were taken before starting the treatment, before repeating the treatment each time at weekly intervals and after the treatment was completed.

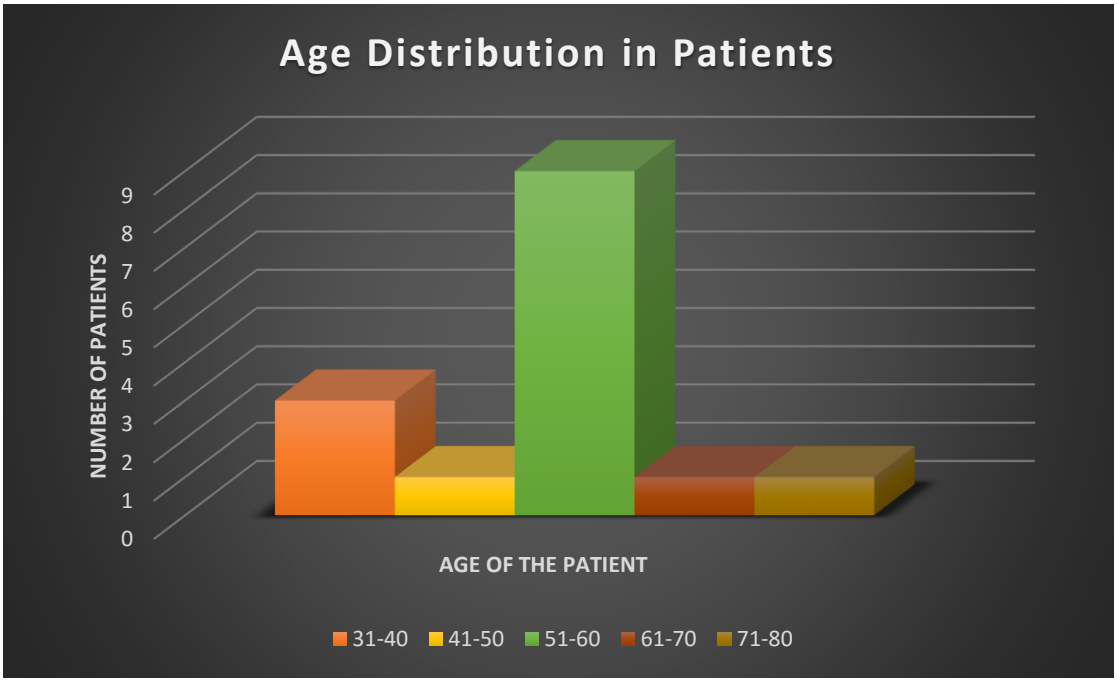
ETHICAL ISSUES INVOLVED IN THE STUDY

- The study involves human subjects with minimal / no risk to them.

RESULTS



In the above conducted study there were 4 female patients (27 %) and 11 male patients (73 %).

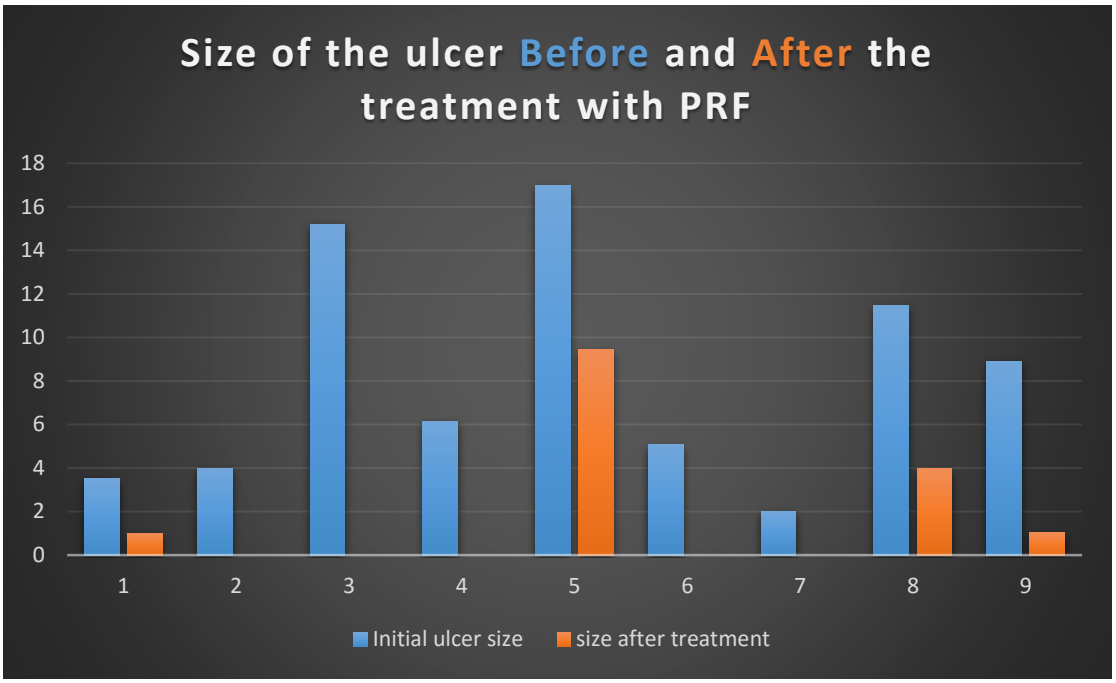


It has been shown in multiple observations that this has been reported more in the older age group. In our study we had

- 9 patients (60 %) in the age group of 51 – 60 years.
- 3 patients (20 %) in the age group of 31 – 40 years.
- 1 patient (6.66 %) in the age group of 41 – 50 years.
- 1 patient (6.66 %) in the age group of 61 – 70 years.
- 1 patient (6.66 %) in the age group of 71 – 80 years.

Patients treated with PRF Dressing						
S. no of patient	Initial measurement in cm^2	Measurement after 1st week	Measurement in cm^2 after 2nd week	Measurement in cm^2 after 3rd week	Final measurement in cm^2	Percentage of improvement
1	**3.5**	**2.64**	**2.64**	**1.0**	**1.0**	**71.42 %**
2	**4**	**3.24**	**1.96**	**0.0**	**0.0**	**100 %**
3	**15.2**	**9.92**	**7.29**	**5.94**	**0.0**	**100 %**
4	**6.16**	**3.57**	**0.0**	**0.0**	**0.0**	**100 %**
5	**16.96**	**16.64**	**13.92**	**11.44**	**9.43**	**44.39 %**
6	**5.06**	**3.80**	**2.24**	**0.0**	**0.0**	**100 %**
7	**2.0**	**1.6**	**1.6**	**0.0**	**0.0**	**100 %**
8	**11.47**	**10.8**	**10.08**	**6.82**	**3.96**	**65.47 %**
9	**8.91**	**3.25**	**1.52**	**1.04**	**1.04**	**88.32 %**
Mean percentage of improvement	**0 %**	**26.27 %**	**46.25 %**	**77.08 %**	**85.51 %**	**85.51 %**

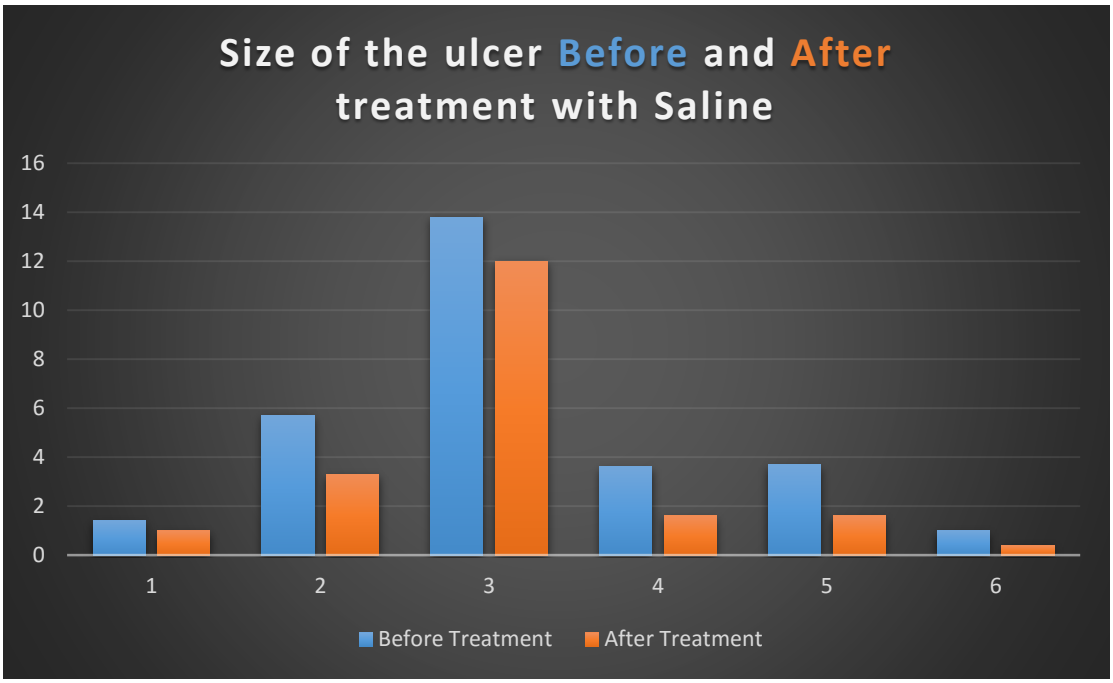
This table shows the 9 patients treated with PRF dressings. With their initial size in cm^2, improvement each week, final measurement and the Percentage of total improvement in each patient and also the mean improvement in each week and overall mean improvement.



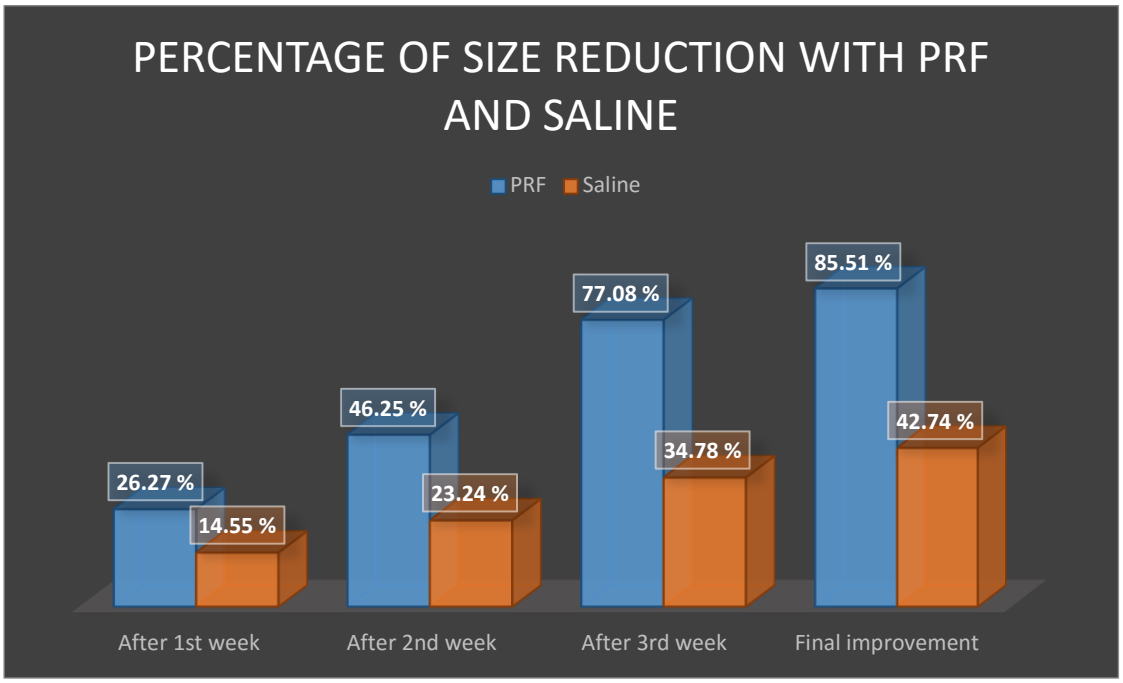
This is the bar diagram of the patients treated with PRF dressing and it shows the reduction of the ulcer size in each patient. In case 2,3,4,6 and 7 we cannot see the bar showing the size reduction because in these cases the ulcer healed completely.

Patients treated with Saline Dressing						
S no of patient	Initial measurement in cm^2	Measurement after 1st week	Measurement in cm^2 after 2nd week	Measurement in cm^2 after 3rd week	Final measurement in cm^2	Percentage of improvement
1	**1.4**	**1.3**	**1.2**	**0.99**	**0.99**	**29.28 %**
2	**5.7**	**4.86**	**4.25**	**4.08**	**3.3**	**42.10 %**
3	**13.8**	**12.6**	**12.6**	**12.0**	**12.0**	**13.04 %**
4	**3.6**	**2.64**	**2.4**	**1.87**	**1.6**	**55.55 %**
5	**3.68**	**2.94**	**2.66**	**2.21**	**1.6**	**56.52 %**
6	**1.0**	**0.9**	**0.7**	**0.5**	**0.4**	**60.00 %**
Mean percentage of improvement	**0 %**	**14.55 %**	**23.24 %**	**34.78 %**	**42.74 %**	**42.74 %**

This table shows the 6 patients treated with saline dressings. With their initial size in cm^2, improvement each week, final measurement and the Percentage of total improvement in each patient and also the mean improvement in each week and overall mean improvement.



This is the bar diagram of the patients treated with Saline dressing and it shows the reduction of the ulcer size in each patient. No ulcer showed complete healing at the end of four weeks.



The bar diagram above shows the comparison of the mean improvement in PRF group and Saline group after every week and also after the treatment for 4 weeks. The group which was treated with PRF showed significant reduction in the ulcer size when compared to the saline group. The mean reduction of ulcer area in the PRF group was 85.51% and the reduction in the saline group was 42.74% at the end of four weeks.

GROUP STATISTICS					
Variables	**No of Patients**	**Mean**	**Std. Deviation**	**t value**	**P value**
Saline	6	42.74	18.52	4.11	<0.001
PRF	9	85.51	20.45		

The above table shows the study was statistically significant with the ‘p value’ of < 0.001.

DISCUSSION

The healing property of tissue forms the basis of surgical treatment. Skin, being one of the 5 senses of the body, its function is not only touch recognition but also to protect the body by functioning as a protective barrier. Any major loss of integrity or continuation of this may lead to exposure of the vital structures present inside the body and could be a cause of morbidity and disability in the form of injury, infection and may occasionally even lead to death.

All wounds which are less than 6 weeks old are called as acute wounds and they heal by a systematic but complicated cascade of molecular and physiologic procedure. But healing is not the same in the wounds which are older than 6 weeks and these wounds are called as chronic wounds [1]. When compared to the acute wounds the healing process in chronic wounds appears to be obstructed somewhere between multiple local and systemic factors. In such cases healing needs a little extra push which is given by the application of platelet rich fibrin. This contains multiple growth factors which are of utmost importance in the initiation and promotion of wound healing which are slowly liberated whenever necessary for rapid and effective healing.

In our study we recruited 15 patients and they were divided in two groups. Group 1 had 9 patients who were treated with weekly PRF dressing for 4 weeks. Group 2 had 6 patients and they were treated with weekly saline dressing for 4 weeks. The group which was treated with PRF showed significant reduction in the ulcer size when compared to the saline group.

In a study conducted by Callam MJ, he stated that varicose veins and venous ulcers are seen more in woman when compared to men. But in our study we got only 27% woman and 73% men suffering with venous ulcers. This could be due to the low sample size in our study.

In a study conducted by David J. Margolis et al. which included 26,599 patients, he concluded that patients who were treated with Products derived by platelets tend to heal faster than patients who were treated without the products derived from platelets. He also concluded that even though the ulcers that were treated with these derivatives were bigger and deeper than the other groups these showed better improvement at the end of 12 weeks[143]. Our study showed similar results where ulcers treated with PRF showed better and faster improvement when compared to saline dressing.

A study carried out by Sean M. O Connell concluded that the treatment of venous and non-venous ulcers had different out comes. He showed that the patients who had venous ulcers and were treated with platelet rich fibrin membrane had a total closure of wound in 66.7 % of patients. Whereas patients who had ulcers which were not of venous origin had total closure in only 44% of patients. Hence he proved that PRF was very efficacious in the treatment of venous ulcers.

In another study Anitua E et al. showed that healing increased significantly with the help of PRF. She also concluded that it not only helps in supplying the required GFs but also by forming fibrin matrix which help in cell migration, it also helps in neo-vascularization.[145] In our study there was a mean reduction of ulcer size of 85.51% in the group treated with PRF at the end of 4 weeks.

In another study Mazzucco er al. concluded that healing is improved and is much faster when the wound is treated with platelet rich gel. He also stated that this also helps reduce the hospital stay. In his study he demonstrated that in patients who were treated with platelet rich gel the wound healed in 3.5 weeks and the wounds which were not treated with platelet rich gel took 6 weeks.[146] In our study 55.55% of patients treated

with PRF had complete closure at the end of 4 weeks. Whereas no patient who was treated with saline had complete closure.

In one more study conducted by G. Saldalamacchia he concluded that the use of platelet rich fibrin had significant effectiveness in the treatment of ulcers. He stated that the use of platelet rich fibrin had better reduction of area of ulcer when compared with the basic wound care without the use of platelet rich fibrin. He also said that though in his study he treated the patients with PRF for a short time it showed good improvement.[147] Similarly in our study we found mean reduction of 85.51% in ulcer area at the end of 4 weeks in patients treated with PRF. Whereas in patients treated with saline dressing showed only 42.74% reduction in the ulcer area.

In a comparative study conducted by Hany Saad Setta et al. he compared the efficacy of platelet rich plasma and the platelet poor plasma in the treatment of diabetic ulcers. In this study he was able to prove that the healing time for the ulcers treated with the platelet rich plasma was much less when compared to the ulcers which were treated with platelet poor plasma.[148]

The efficacy of the platelet rich gel was also shown by another study which was conducted by Gino Bernuzzi et al. which showed that platelet gel reduced the duration of healing. He proved that there was a significant effect on the cell proliferation which helped greatly in healing. He also stated that this effect was dose dependent.[149]

In a randomised study which was conducted by Steed DL et al. he proved that the use of activated platelet supernatant in the treatment of diabetic ulcers had much better results in complete healing and closure of the ulcer when compared to a placebo which had only saline. In his study he got 94% closure and healing rate at the end of 20 weeks with the platelet supernatant and 73 % closure in the placebo group. He concluded that the platelet supernatant was useful in reducing the downtime required in the treatment of chronic ulcers.[66] In our study out of the 5 who showed complete closure with PRF dressing 1 patient achieved closure in two weeks, 3 achieved closure after 3 weeks and 1 achieved closure after 4 week.

In many studies, there was no significant improvement in the ulcers treated with saline dressing. [143, 146,147, 66] Our study also showed similar results.

CONCLUSION

In our study, there was high ulcer reduction rate at the end of 4 weeks in the patients treated with PRF dressings when compared to the saline group, which was statistically significant. As this procedure is effective, simple, patient friendly, cost effective, painless and can be done as an outpatient procedure, we would like to conclude that the use of Platelet Rich Fibrin dressings as an adjuvant therapy in the treatment of chronic venous ulcer is effective, as it shows great potential to achieve complete closure of ulcers and can successfully be used as a routine procedure in the treatment of venous ulcers.

Limitations

- Small sample size.

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CLINICAL PHOTOGRAPHS

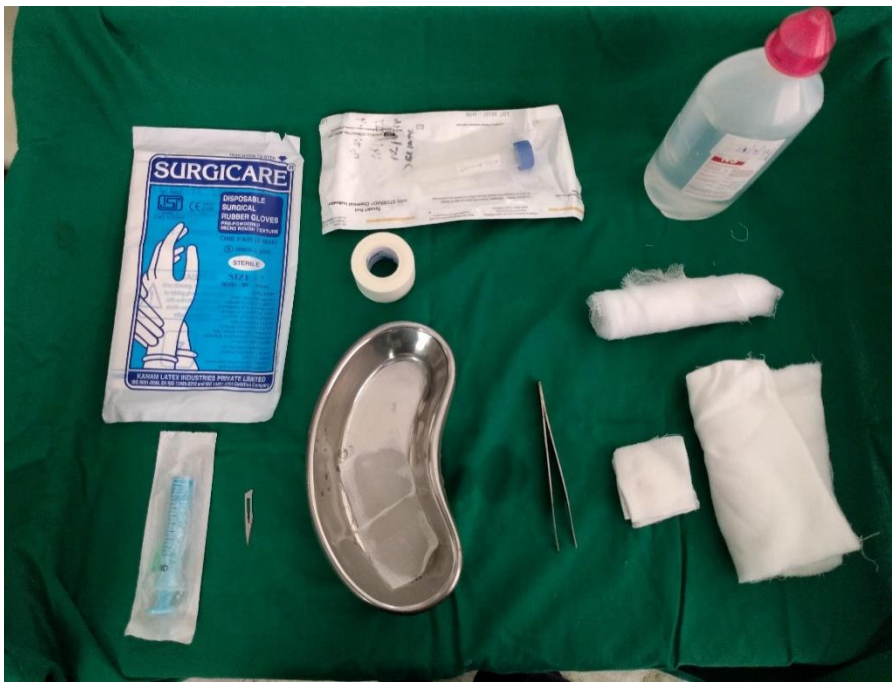
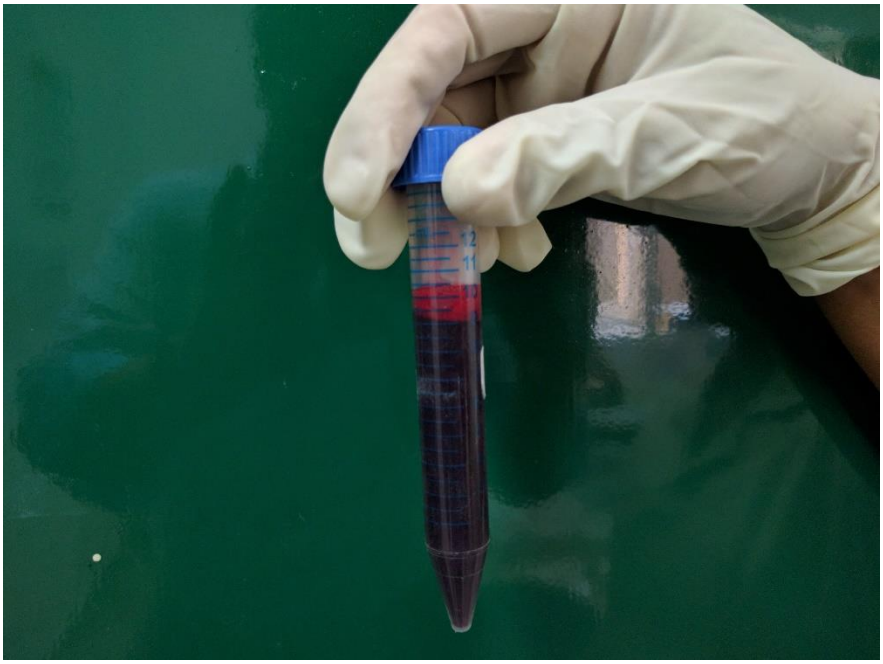


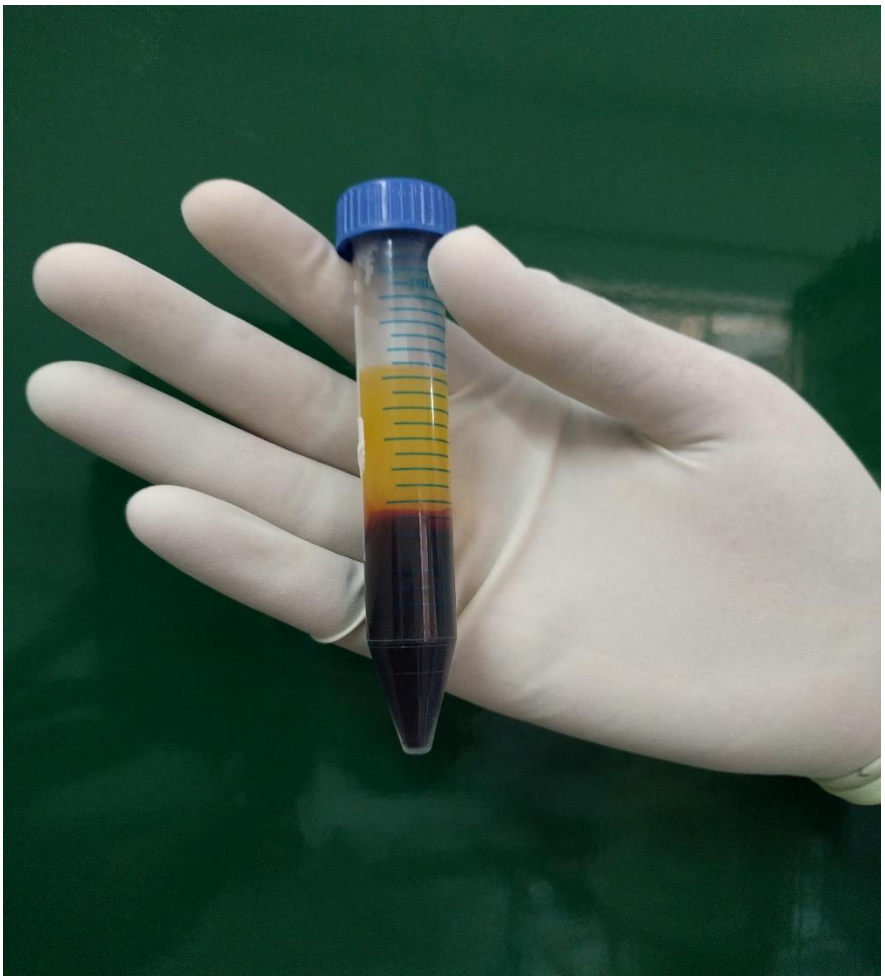
Figure -1

Figure -2

PROCEDURE

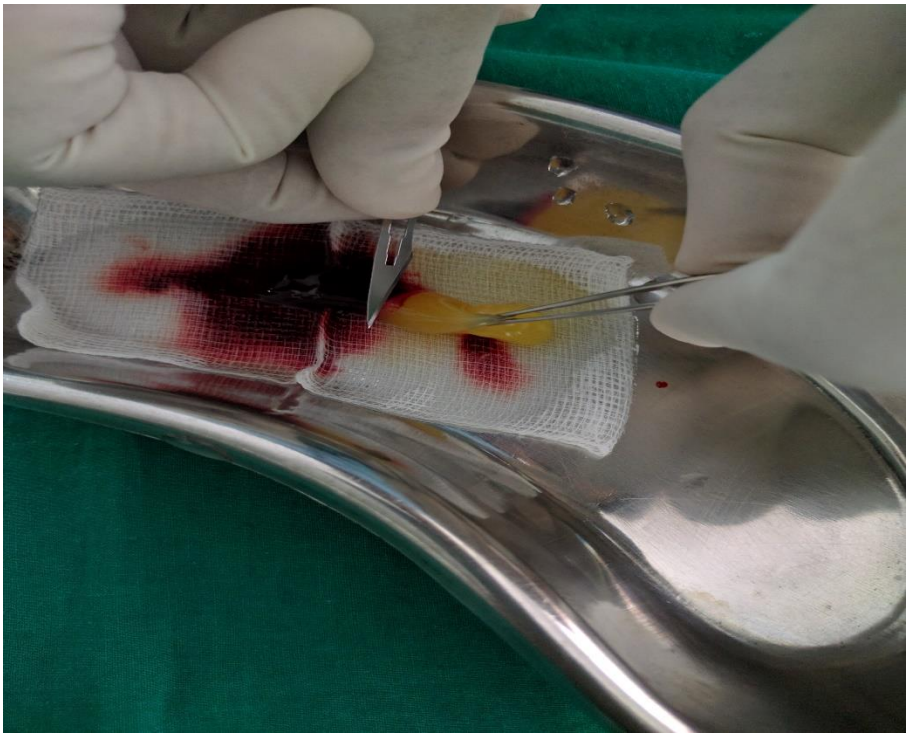


10 ml BLOOD IN VACUTAINAER

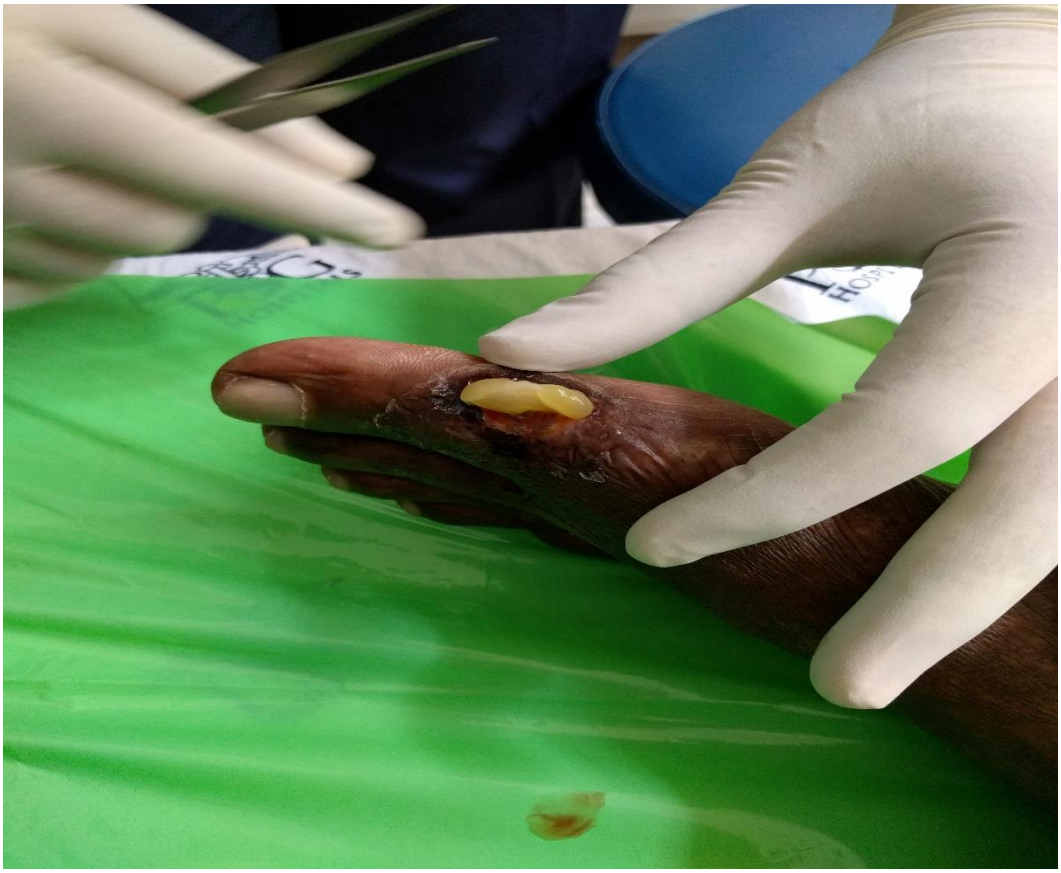


PRF CLOT

REMOVE PRF CLOT WITH NON TOOTHED FORCEPS



SCRAPE – OFF ADHERENT RBC’s



PLACE THE PRF CLOT OVER THE ULCER

COVER THE ULCER WITH STERILE GAUZE PIECE
(PRIMARY DRESSING)

COVER THE ULCER WITH STERILE GAUZE PAD
(SECONDARY DRESSING)

PATIENTS TREATED WITH PRF DRESSING

BEFORE

AFTER

BEFORE

AFTER

BEFORE

AFTER

BEFORE

AFTER

BEFORE

AFTER

PATIENTS TREATED WITH SALINE DRESSING

BEFORE

AFTER

BEFORE

AFTER

BEFORE

AFTER

CONSENT FORMS

PSG Institute of Medical Science and Research, Coimbatore
Institutional Human Ethics Committee
INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

I Anirudh Somani am carrying out a study on the topic: "Efficacy of autologous **platelet rich fibrin (PRF)** over moist sterile saline dressing in **chronic venous leg ulcers** as part of my research project being carried out under the aegis of the Department of DERMATOLOGY VENEROLOGY & LEPROLOGY.

My research guide is: Dr. Reena Rai

The justification for this study is: Chronic venous leg ulcers is a result of the progression of chronic venous insufficiency. This often affects the quality of life of patients and shows a protracted healing, resulting in economic burden. Cutaneous wound healing involves release of platelet growth factors and chronic wounds have been hypothesized to be deficient in them. It has been suggested that topical administration of platelet derivates, including "platelet-rich plasma" and "platelet gel" can enhance tissue repair. Platelet derivates have been used to accelerate tissue repair in orthopaedic surgery, dental surgery, plastic surgery, and chronic diabetic ulcers. Topical platelet derivates have been found to be efficacious in enhancing tissue repair, including cutaneous ulcers of varied etiology, in randomized control trials.

The objectives of this study are:

Primary Objective: To study the efficacy of autologous **platelet rich fibrin (PRF)** in **chronic venous leg ulcers over moist saline dressing.**

Sample size: 20

Study volunteers / participants who present with venous ulcers to the Dermatology OPD.

Location: PSG IMSR

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Initial interview (specify approximate duration): 15 minutes.

Data collected will be stored for a period of 10 years. We will / will not use the data as part of another study.

Blood sample collection: Specify quantity of blood being drawn: 10ml.

No. of times it will be collected: 4 times over one month (weekly once)

Whether blood sample collection is part of routine procedure or for research (study) purpose: Routine procedure
Specify **purpose**, discomfort likely to be felt and side effects, if any: nil

Whether blood sample collected will be stored after study period: No, it will be destroyed

Whether blood sample collected will be sold: Yes/**No**

Whether blood sample collected will be shared with persons from another institution: Yes/**No**

Final interview (specify approximate duration): 10 minutes. If **photograph** is taken, purpose: To compare improvement in the size of ulcer

Benefits from this study: This procedure could be used to treat chronic non healing ulcers.

Risks involved by participating in this study: nil

How the **results** will be used: Results will be compared to find the efficacy of PRF.

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at any time.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date:
Witness:
Contact number of PI: 9500989789
Contact number of Ethics Committee Office: 0422 2570170 Extn.: 5818
ஒப்பதல் படிவம்

தேதி

அனிருத் சொமானி ஆகிய நான் PSG மருத்துவக்கல்லூரியின் **தோல் பால்வினை மற்றும் தொழுநோய்** துறையின் கீழ் "**இரத்தக் குழாயில் ஏற்பட்ட பாதிப்பினால் உண்டான நாற்பட்ட புண்ணிற்கு, இரண்டு விதமான சிகிச்சை முறைகளான சலைன் டிரஸ்ஸிங் மற்றும் தட்டை அணுக்கள் நிறைந்த ∴வைப்ரின் டிரஸ்ஸிங் ஆகிய இவற்றுள் ஏது சிறந்த மருத்துவ சிகிச்சை என்பதை கண்டறிதல்**" என்ற தலைப்பில் ஆய்வு மேற்கொள்ள உள்ளேன்

என் ஆய்வு வழிகாட்டி : **மரு.ரீனா ராய்**

ஆய்வு மேற்கொள்வதற்கான அடிப்படை:

இரத்தக் குழாயில் பாதிப்பு ஏற்பட்டால் அதனால் வரும் புண் நீண்ட நாள் ஆறாமல் இருக்கும். இந்த புண்ணை குணப்படுத்த பல்வேறு சிகிச்சை முறைகள் இருக்கின்றன. அவற்றுள் சலைன் டிரஸ்ஸிங் மற்றும் தட்டை அணுக்கள் நிறைந்த ∴வைப்ரின் டிரஸ்ஸிங் ஆகிய இரண்டு மருத்துவ சிகிச்சைகள் உள்ளன. இந்த இரண்டு சிகிச்சை முறையில் எது சிறந்தது என்று அறிதல்.

ஆய்வின் நோக்கம் :

இரத்தக் குழாயில் பாதிப்பு ஏற்பட்டால் அதனால் வரும் புண்ணை சிறந்தமுறையில் குணப்படுத்துதல்

ஆய்வு மேற்கொள்ளும் இடம் :

பி.எஸ்.ஜி மருத்துவமனை,
தோல் பால்வினை மற்றும் தொழுநோய் துறை

பரிசோதனைக்கு உட்படுத்தப்பட்டவர்களின் எண்ணிக்கை : 15

வயது வரம்பு : 18 வயதிற்கு மேல்

இடம் : தோல், பால்வினை மற்றும் தொழுநோய்துறை
பி.எஸ்.ஜி மருத்துவமனை, கோயம்புத்தூர்

இந்த ஆய்வுக்கு எங்களுக்கு ஒத்துழைப்பைத் தருமாறு தங்களைக் கேட்டுக் கொள்கிறோம். மேலும் இந்த ஆய்வுக்குத் தேவையான தகவல்கள் உங்களிடமிருந்து பெற்றுக் கொள்ளப்படும்.

பரிசோதனையின் போது ஏற்படும் பக்கவிளைவுகள் தோல் : இல்லை

புகைப்படம் எடுக்கப்பட்டதா? அதன் நோக்கம் :

பரிசோதனைக்கு முன்பும், பின்பும் உள்ள மாற்றத்தை ஒப்பிட

இந்த ஆய்வில் பங்கேற்க ஒப்புக் கொள்ளுவதால் எந்தவிதமான பலனும் உங்களுக்கு கிடைக்காது. எந்த நேரத்தில் வேண்டுமானாலும் ஆய்விலிருந்து விலகிக் கொள்ளும் உரிமை உங்களுக்கு உண்டு.

ஆய்விலிருந்து விலகிக் கொள்வதால் உங்களுக்கு அளிக்கப்படும் சிகிச்சையில் எந்த வித மாற்றமும் இருக்காது.

மேலும் இந்த ஆய்வில் பங்கு கொள்வது உங்கள் சொந்த விருப்பம். இதில் எந்த விதக் கட்டாயமும் இல்லை. நீங்கள் விருப்பப்பட்டால் இந்த ஆய்வின் முடிவுகள் உங்களுக்குத் தெரியப் படுத்தப்படும்.

ஆய்வாளரின் கையொப்பம் :

தேதி :

ஆய்வுக்குட்படுபவரின் ஒப்புதல்

நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் அதன் பயன் பாட்டினைப் பற்றி தெளிவாகவும் விளக்கமாகவும் தெரியப்படுத்தப்பட்டுள்ளேன். இந்த ஆராய்ச்சியல் பங்கு கொள்ளவும் இந்த ஆராய்ச்சியின் மருத்துவ ரீதியான குறிப்புகளை வரும் காலத்திலும் உபயோகப்படுத்திக் கொள்ளவும் முழு மனதுடன் சம்மதிக்கிறேன்.

ஆய்வுக்குட்படுபவரின் பெயர், முகவரி :

கையொப்பம் :

தேதி :

PROFORMA

Variables Recorded

- Name: Age: Gender: Phone no:
- Op / Ip no:
- Address:
- Occupation:
- Complaints:

- Duration of the ulcer:
- Diagnoses:
- Venous Doppler: Yes / No

- Ulcer bed:
- Measurement: L / B / D / DATE
  Initial :
  1st week:
  2nd week:
  3rd week:
  Final measurement:

- Infection: Yes / No
- Co-morbidities: HTN / BA / DM
- Blood counts: Platelets- Hb-
- Previous treatment (if any):
  Dressing
  Drugs
  Topical

MASTER CHART

MASTER CHART

S No of patient	Initial Measurement in cm^2	Measurement after 1st week in cm^2	% Reduction in size after 1st week	Measurement after 2nd week in cm^2	% Reduction in size after 2nd week	Measurement after 3rd week in cm^2	% Reduction in size after 3rd week	Final Measurement in cm^2	Final reduction in %
PRF DRESSINGS									
1	3.5	2.64	24.57	2.64	24.57	1.0	71.42	1.0	71.42
2	4	3.24	19	1.96	51	0	100	0	100
3	15.2	9.92	34.73	7.29	52.03	5.94	60.92	0	100
4	6.16	3.57	42.04	0	100	0	100	0	100
5	16.96	16.64	1.88	13.92	17.92	11.44	32.54	9.43	44.39
6	5.06	3.80	24.90	2.24	55.73	0	100	0	100
7	2.0	1.6	20	1.6	20	0	100	0	100
8	11.47	10.8	5.84	10.08	12.11	6.82	40.54	3.96	65.47
9	8.91	3.25	63.52	1.52	82.94	1.04	88.32	1.04	88.32
Mean Improvement			26.27		46.25		77.08		85.51
SALINE DRESSINGS									
10	1.4	1.3	7.14	1.2	14.28	0.99	29.28	0.99	29.28
11	5.7	4.86	14.73	4.25	25.43	4.08	28.42	3.3	42.10
12	13.8	12.6	8.69	12.6	8.69	12	13.04	12	13.04
13	3.6	2.64	26.66	2.4	33.33	1.87	48.05	1.6	55.55
14	1.0	2.94	20.10	2.66	27.71	2.21	39.94	1.6	56.52
15	1.0	0.9	10	0.7	30	0.5	50	0.4	60
Mean Improvement			14.55		23.24		34.78		42.74

ABBREVIATIONS

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PRF	Platelet Rich Fibrin
CVI	Chronic Venous Insufficiency
TNF- α	Tumour Necrosis Factor Alpha
F-β	Transforming Growth Factor Beta
GF	Growth Factor
EGF	Epidermal Growth Factor
TGF- α	Transforming Growth Factor Alpha
GI	Gastro Intestinal
IGF-I	Insulin-like growth factor-I
RNA	Ribo-Nucleic Acid
PDGF	Platelet Derived Growth Factor
Bfgf	Basic Fibroblast Growth Factor
PRP	Platelet Rich Plasma
PRFM	Platelet Rich Fibrin Membrane
OPD	Out-Patient Department